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Proceedings of the Fourth Symposium on Virus Diseases of Fruit Trees in Europe

Lyngby 25.—30. July 1960

Fourth Symposium on Virus Diseases of Fruit Trees in Europe

1. J. A. Milbrath U. S. A.
2. G. Nyland U. S. A.
3. E. Gram Denmark
4. J. W. Blencowe England
5. K. S. Bhargava India
6. A. Attafuah Ghana
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11. C. H. Cadman Scotland
12. Z. Hidaka Japan
13. J. R. Shay U. S. A.
14. S. Dalbro Denmark
15. K. Persson Denmark
16. L. Kunze Germany
17. H. Kegler D. D. R.
18. Chr. Stapel Denmark
19. G. Vukovits Austria
20. R. Cropley England
21. S. Blumer Switzerland
22. A. F. Posnette England
23. G. Schmid Switzerland
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27. A. Corte Italy
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29. K. Schuch Germany
30. R. Bovey Switzerland
31. D. J. Engelbrecht S. Africa
32. H. Dias Portugal
33. O. Canova Italy
34. N. F. Buchwald Denmark
35. P. Remy France
36. R. Bernhard France
37. M. Christensen Denmark
38. N. Paludan Denmark
39. A. Thomsen Denmark
40. H. Rønde Kristensen Denmark
41. G. L. Stout U. S. A.
42. E. Tapio Finland
43. G. Bauman Germany
44. H. J. Pfaeltzer Holland
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48. M. Jordovic Jugoslavia
49. L. D. Wolfswinkel S. Africa
50. E. Larsen Denmark
51. F. Gualaccini Italy

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5. K. S. Bhargava	India	22. A. F. Posnette	England	39. A. Thomsen	Denmark
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15. K. Persson	Denmark	32. H. Dias	Portugal	49. L. D. Wolfswinkel	S. Africa
16. L. Kunze	Germany	33. O. Canova	Italy	50. E. Larsen	Denmark
17. H. Kegler	D. D. R.	34. N. F. Buchwald	Denmark	51. F. Gualaccini	Italy

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Compiled by H. Rønde Kristensen

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Introductory Note

Since the first symposium on virus diseases of fruit trees in Europe in 1954 was held in Switzerland, very much work has been done on fruit tree viruses all over the world and during the following symposia in the Netherlands (1955) and in England (1956) valuable information were presented by several workers, which undoubtedly was a great stimulation for all the future work in this particular field of virology.

When assembled at East Malling Research Station in 1956 the participants kindly accepted the invitation to have the fourth symposium in Lyngby, Denmark, and during a committee meeting it was decided that the symposium should take place in the period 25th-30th July, 1960.

Invitation to participate were in September 1959 sent to colleagues in the European countries as well as to some "observers" from America, Africa, Asia, and Australia, and we were greatly pleased with many positive answers.

Unfortunately a few registered members were not able to come, but even so many countries were represented, and the total number of participants were 52.

34 papers are included in this publication together with the main points of the discussions held after each.

In Lyngby we were happy to organize the symposium and to welcome so many colleagues from abroad, and we wish to thank everybody for the most kind and willing collaboration during the meetings, which we hope will contribute to the already existing cooperation and understanding between the fruit tree virus workers.

We also wish to acknowledge with thanks the financial support from the Danish Ministry of Agriculture and from the National Committee for the Propagation and Sanitary Inspection of Horticultural Plants.

Furthermore, thanks are due to the Editional Board of Tidsskrift for Planteavl, for all the work connected with the publication of this proceedings.

On behalf of the organizing committee

H. Rønde Kristensen

Opening address

Ernst Gram

If we could listen to two doctors discussing, one hundred years ago, their cases of consumption we might admire their ability of diagnosis, their attention to aggravating factors, their experience in prognosis. We would with great understanding hear their opinions on causality – a score of years before *Bacillus tuberculosis* and its fatal role was discovered – we who only 50 years ago were in the same situation when discussing e.g. potato leaf-roll. You will remember that this abnormality was first recognized as a particular plant disease when, in 1905, the German pathologist O. Appel participated in an excursion here in Denmark.

In the radiant days of July, in the year 1914, the Danish Plant Protection Service arranged its first course in potato field inspection here in Lyngby. Damp from the press we received professor Kølpin Ravn's manual on plant diseases, with pages on potato leaf-roll and on mosaic of tobacco, potato, and beets. Participants were the agricultural advisers and one inexperienced student of botany, who found himself, a week later, confronted with and expected to solve the riddles of potato fields. Soils were light and dry, muriate of potash had been distributed late in spring, conscientious farmers had destroyed many rootlets by too energetic cultivation, and therefore in a warm and dry summer rolling of leaves presented the most varying and puzzling aspects.

If the marks given for potato leaf-roll in 1914 had been followed up by cultivation of clones in the following year the assistant inspector might have been asked politely to hand in his resignation. The knot, however, was dealt with in a Gordian way, world events of 1914 transferring the concern from seed potatoes to an acute demand for table potatoes. Still, an interest in the effect of soil, climate, and nutrition on disease symptoms was stressed. Abundant objects of study were found at the State

Experimental Stations which were distributed on different soil types, and which presented climatic differences evidently large enough to be of importance.

Differing and rather confusing information concerning growth conditions as the cause of leaf-roll and other "degeneration" of the potato plants were presented during these years. The founder of our Phytopathological Institute, professor Kølpin Ravn, therefore, carried out an experiment, selecting one healthy and one leaf-rolling lot of Magnum Bonum, which were distributed to 12 experimental stations and grown there in the years 1915 to 1919. Each autumn, samples were sent to this part of Sealand and in the following spring placed in a field experiment in such a way that the course of leaf-roll percentage and of tuber production during the 5 years could be determined. Here in Lyngby, a classic locality of leaf-roll, the healthy lot had only 4 per cent of healthy plants left after 5 years, 96 per cent showing distinct leaf-roll in northern Jutland the same healthy lot after 5 years showed 1-2 per cent leaf-roll, while the diseased lot appeared to have recovered, only 1-4 per cent of plants with leaf-roll remaining. This apparent recovery represents one of those cases where summary treatment of numbers contributes to the doubtful reputation of statistics. When no new infection takes place, when each diseased plant produces 2 seed potatoes, and each healthy plant 10, then the diseased part of the population will, of course, soon be crowded out.

It became my duty to write the report on this experiment. In 1921 The Royal Horticultural Society and the Ministry of Agriculture invited those interested to a potato conference and exhibition in London. I was interested and brought in my pocket a map showing how the potatoes recovered on the light soils in northern and western Denmark. When I showed it to professor H. M. Quanjer he said that in the Netherlands, too, the potatoes were healthy in the north and west – but here the soils were clayey, while the bad potato region in southeast was sandy. Two days later I saw in Wageningen the convincing effect of aphids admitted to or kept away from potatoes! Certainly, Allard had in 1914 in U.S.A. pointed to insects as vectors of mosaic, and Lind had here in Lyngby at the same time indicated *Aphis fabae*

as vector of beet mosaic – but it was professor Quanjers unravelling of the potato virus tangle that initiated a new epoch.

I regret, before the present illustrious company of specialists in fruit tree viroses, to have to select illustrations from crops like beets and potatoes. But when the terms virus and virosis came to be widely applied in plant pathology, trees were not generally considered as possible hosts to virus (to put it mildly). Working with several ornamentals with albicatio and other irregularities Lindemuth reports in 1878 (Thiels Landw. Jahrbücher H.6.) on transmission by grafting, but ascribes the results to a vegetative bastardization. 40 years later Hesler & Whetzel in their Manual of Fruit Diseases (1917) give som pages on peach yellows, declaring, in the very heading, the cause to be unknown. They list a number of ideas ventured since 1760, when peach yellows began to be troublesome in Pennsylvania, and conclude that opinions now tend towards an unknown parasite. The manual is representative of what was taught in America during those years. But Erwin F. Smith, reporting already in 1890-91 on his positive results of grafting experiments with peach yellows is cited as having used those very words "some sort of virus" quite natural to him as a bacteriologist. Erwin F. Smith has certainly not meant bacteria and bacterial toxins, being one of the pioneers of bacterial plant pathology, and he makes no mention at all of peach yellows in his stately books on bacterial plant diseases – he was using the term virus in a noncommittal albeit expectant way.

Plant pathologists, still burdened by a Latin conscience, hesitated, wavering between vira and viruses – it must be admitted that the lacking plural of the Latin word, as numbers of viroses describe multiplied, proved an unforeseen deficiency.

Not only the word virus was objected to. In the first edition of P. Sorauer's Handbuch der Pflanzenkrankheiten (1874) we find very few phenomena which would now be considered viroses of ligneous plants (a mosaic in vine? some ornamentals) in the second edition (1886) likewise, and in the third edition in 1909 viruses were swept aside with the words "Wir brauchen also gar nicht die Annahme eines Virus . . .".

Of trees, Conifers and other Gymnosperms are still considered free from viroses, I believe, but for how long? The temptation is

great to explore farther down, among spore plants. I wonder what is the status among the green algae? Whether one-celled, threads, or subtle fronds, they are so self-revealing under the microscope, cultivable, studied intensively by plant physiologists -- might they not contribute to fundamental virological study?

With wishes for a deeper insight into the nature of virus, for increase in the valuable assistance already offered to plant culture, but not exactly for more viroses, I have the honour and pleasure of declaring this 4th Symposium on Virus Diseases of Fruit Trees in Europe open.

Die Anfälligkeit verschiedener Apfelsorten gegenüber dem Apfelmosaikvirus

G. Hamdorf

Obstbauversuchsanstalt Jork,
Altes Land, Bez. Hamburg, Bundesrepublik Deutschland

Zu den am stärksten verbreiteten Kernobstvirosen des Niederelbgebietes zählt des Apfelmosaik (Pyrus virus 2 (Bradford et Joley) Smith, Marmor mali Holmes), welches teils sporadisch, teils in grösserem Umfange in unseren Ertragsanlagen und Baumschulquartieren auftritt. Stärkere Vorkommen dieser Virose konten sowohl bei Junganlagen als auch bei älteren Baumbeständen ermittelt werden.

Eindeutige Symptome dieser Virose wurden bisher 18 Sorten unseres Anbaugebietes in mehr oder minder starker Ausprägung festgestellt, so z.B. bei Altländer Pfannkuchen, Coulon Renette, Cox Orange Renette, Echter Glockenapfel, Finkenwerder Herbstprinz, Fettapfel, Golden Delicious, Goldparmäne, Grahams Jubiläum, Gravensteiner, Holsteiner Cox, Horneburger Pfannkuchen, Ingrid Marie, James Grieve, Jonathan, Laxton's Superb, Schöner von Boskoop und Schurapfel. Vielfach ist jedoch die Ausprägung des Symptombildes sehr schwach, und bei manchen Sorten wie Krügers Dickstiel, Transparent von Croncels und Weisser Klarapfel wurden bisher niemals Mosaiksymptome beobachtet.

In welchem Masse die Ausprägung der bei den einzelnen Sorten beobachteten Symptome auf Sorteneigenschaften der Wirtspflanze oder nach Posnette und Cropley (1953/56) auf das Vorkommen verschiedener Stämme des Apfelmosaikvirus zurückzuführen ist, liess sich mangels entsprechender Untersuchungen für unser Sortiment bisher nicht entscheiden. Auch die Kentnisse über eine mögliche Toleranz der in unserem Anbau befindlichen Sorten sind noch unzureichend.

Erste Ergebnisse bezüglich dieser Fragestellung liegen von Kegler (1959) vor, der die Anfälligkeit einzelner, in der DDR

vorkommender Apfelsorten und Malusformen gegenüber einer Herkunft des Apfelmosaikvirus untersuchte und einen unterschiedlichen Toleranzgrad bei denselben feststellte.

Der weiteren Klärung dieser Frage dienten eigene Untersuchungen, die in den Jahren 1958 bis 1960 an der Obstbauversuchsanstalt in Jork durchgeführt wurden. Berücksichtigung fanden dabei einerseits Sorten, die im Bundessortiment sowie in dem des Niederelbischen Anbaugebietes vertreten sind, und andererseits solche, welche bereits von anderen Untersuchungen benutzt und zum Vergleich herangezogen wurden.

Der vergleichenden Prüfung unterlagen ferner unveredelte Apfelunterlagen der Typen MM 104, MM 109, MM 111, EM II, EM IV, EM VII, EM IX, EM XI, EM XVI, EM XXV und Crab C.

Zur Prüfung der Anfälligkeit verschiedener Apfelsorten gegenüber dem Apfelmosaikvirus wurden im Frühjahr 1958 jeweils 10 Pflanzen des Typs EM VII mit 38 verschiedenen Apfelsorten (insgesamt 380 Veredlungen) veredelt und im Freiland angezogen. Bei der Sorte Sunset starben zahlreiche Veredlungen ab, sodass 37 Sorten in der endgültigen Testung standen.

Sämtliches Reisermaterial der zur Veredlung von Typ VII benötigten Sorten stammte aus der betriebseigenen Versuchsanlage in Ottensen, wobei stets nur ausgesuchte, seit mehreren Jahren auf sichtbaren Virusbefall geprüfte Bäume als Reiserlieferanten dienten. Ausserdem konnte bei der im Jahre 1959 durchgeführten Testung (Indikatorpflanze: Grahams Sämling) in keinem Falle eine latente Verseuchung mit dem Apfelmosaikvirus bei den im Versuch stehenden Sorten nachgewiesen werden.

Die Infektion des Pflanzenmaterials erfolgte Anfang Mai 1958 nach der in England geübten Methode der Schildchenpfropfung (chip-budding), indem jeweils 5 der Versuchspflanzen einer Sorte mit je einem Rindenschildchen von 2,5 cm Länge, welches von einem mosaikkranken Baum der Sorte Jonathan stammte, versehen wurden, während die übrigen 5 Pflanzen jeder Parzelle als Kontrollen verblieben. Zur Pfropfung fanden nur Rindenschildchen Verwendung, die aus Zonen sichtbar erkrankter Zweige vom vorjährigen Holz stammten, da für das Apfelmosaikvirus wiederholt nachgewiesen werden konnte (Luckwill, 1953; Posnette und Cropley, 1956), dass es nicht vollsystemisch ist.

Da bereits in Vorversuchen geklärt war, dass die Unterlage EM VII sehr starke Symptome bei Infektion mit der vorhererwähnten Virusherkunft zeigt, wurden die Unterlagen nach der Veredlung nur unvollständig aufgeputzt und einzelne Tribe zur Kontrolle einer erfolgten Infektion an der Pflanze belassen.

Nach einer Inkubationszeit von 14 Wochen zeigten sich die ersten Symptome dieser Virose an einzelnen Pflanzen der verschiedenen Sorten und Unterlagentypen; im folgenden Jahr wiesen jedoch sämtliche Sorten Mosaiksymptome in unterschiedlicher Ausprägung und Stärke auf, während die Kontrollen symptomlos blieben.

Die Ergebnisse 3-jähriger Beobachtungen wurden in den Tabellen 1 und 2 zusammengestellt, wobei die Einordnung der Sorten nach ihrer Anfälligkeit gegenüber dem Apfelmosaikvirus erfolgte. Der Anfälligkeitswert stellt dabei mit wenigen Ausnahmen jeweils einen Mittelwert aus 5 Untersuchungen (5 Pflanzen) dar und variiert zwischen 14,4 und 0,2 von den stark anfälligen bis zu den schwach anfälligen Sorten. Bei der Bonitierung fanden sowohl der Anteil symptomtragender Blätter als auch die Stärke des Symptombildes des Einzelblattes Berücksichtigung. Das Produkt beider Werte ergab den Anfälligkeitswert.

Von den Edelsorten (s. Tabelle 1) wiesen Golden Delicious, Roter Martini, Jonathan und Taunton Cross die stärkste Anfälligkeit auf (14,4–12,0), während die Sorten Miller's Seedling, Weisser Klarapfel, Jacob Fischer und Krügers Dickstiel am schwächsten reagierten (0,8–0,2).

Bei den zuerst genannten Sorten traten die Symptome als kleine oder grössere, gelbe bis gelbgrüne Flecken sowie als Ring- und Bandmuster in Erscheinung, welche die Blattspreite mehr oder weniger stark bedeckten und an fast allen Trieben der Versuchspflanzen zu finden waren. Bei den übrigen Sorten war ein ähnliches Symptombild zu beobachten; lediglich die Befallsstärke der einzelnen Blätter und Triebe war geringer. Teilweise traten auch Gelbfärbungen im Bereich der Blattadern auf. Sorten mit schwacher Anfälligkeit wie Miller's Seedling, Weisser Klarapfel, Jacob Fischer und Krügers Dickstiel zeigten schliesslich nur noch geringe hellgrüne bis gelbgrüne, punktförmige Aufhellungen, die sporadisch an einzelnen Blättern zu finden waren.

Tabelle 1. Die Anfälligkeit verschiedener Apfelsorten gegenüber dem Apfelmosaikvirus

Sorten mit starker Anfälligkeit	Anfälligkeitswert
Golden Delicious	14.4
Roter Martini	13.8
Jonathan	13.8
Taunton Cross	12.0
Sorten mit mittlerer Anfälligkeit	
Tydeman's Late Orange	11.7
Bramley's Seedling	9.9
Cox Orange Renette	9.9
Winston	9.6
Rival	8.4
Grahams Jubiläum	8.4
Allington Pepping	8.4
Laxton's Epicure	7.5
Tydeman's Early Worcester	7.2
Worcester Pearmain	6.6
Melba	6.6
Echter Glocken	6.6
Sorten mit schwacher Anfälligkeit	
Goldparmäne	4.0
Blenheim Orange	4.0
Horneburger Pfannkuchen	3.6
Newton Pepping	3.2
Ellison's Orange	3.2
Finkenwerder Herbstprinz	3.2
Geheimrat Oldenburg	3.0
Coulon Renette	2.8
Brettacher Sämling	2.4
Schöner aus Bath	2.4
Ingrid Marie	2.0
Transparent	2.0
Zabergäurenette	2.0
Gravensteiner	2.0
James Grieve	1.6
Schöner von Boskoop	1.6
Early Victoria	0.8
Krügers Dickstiel	0.7
Jacob Fischer	0.3
Sorten mit sehr schwacher Anfälligkeit	
Weisser Klarapfel	0.2
Miller's Seedling	0.2

Bonitierungsschema zu Tabelle 1:

a) Anteil symptomtragender Blätter:
   1 = 1 — 5 %
   2 = 5 — 10 %
   3 = 10 — 25 %
   4 = 25 — 50 %
   5 = 50 % und mehr

b) Stärke des Blattbefalls:
   1 = schwach (einzelne kleine hellgrüne bis gelbgrüne Flecke),
   2 = mittel (einzelne grössere gelbe Flecke, Ring- und Bandmuster),
   3 = stark (gelbe Blattfleckung, die einen grösseren Teil der Blattspreite bedeckt, Ring- und Bandmuster, gelbgefährte Blattzonen z.T. nekrotisch werdend)

Von den Unterlagentypen wiesen EM XXV und Crab C die stärkste Anfälligkeit auf, während EM IV, EM II, MM 111 und EM XVI nur sehr schwache und vereinzelt auftretende Symptome entwickelten (s. Tabelle 2). Charakteristisch für die schwach anfälligen Unterlagentypen waren punktförmige, hellgrüne Verfärbungen der Blattspreite; bei den übrigen vegetativen Unterlagen herrschten gelbgrüne Flecken, verschiedenen Ausmasses sowie ring- und bandförmige Muster vor, die bei Typ EM VII besonders gut ausgeprägt waren. Crab C zeichnete sich ferner durch grossflächige Chlorophylldefekte und Blattverfärbungen im Bereich der Blattadern aus.

Die in Tabelle 2 aufgeführte Rangordnung nach der Anfälligkeit der verschiedenen Unterlagentypen stimmt im allgemeinen mit der von Posnette und Cropley (1956) aufgestellten überein. In der Ausbildung der ring- und bandförmigen Blattmuster, die bei Typ EM VII charakteristisch und besonders gut ausgebildet waren, und der geringen Symptomausprägung bei den EM Typen IV, II, XVI und MM 111 ergibt sich ferner eine gute Übereinstimmung des eigenen Mosaikstammes mit dem von Posnette als »intermediate« oder »mild vein-banding strain« bezeichneten Stammes; vielleicht ist er mit diesem identisch.

Auch die bei den englischen Edelsorten (s. Tabelle 1) ermittelten Anfälligkeitswerte zeigen mit den von Luckwill (1953) sowie von Posnette und Cropley (1956) gewonnenen Ergebnissen eine relativ gute Übereinstimmung. Auffällig ist jedoch, dass Luckwill bei Infektion mit dem »vein-banding strain« eine Toleranz bei den

Sorten Ellison's Orange und Rival feststellte, während in den eigenen Versuchen die Sorte Rival relativ stark reagierte und Ellison's Orange noch eine schwache Anfälligkeit aufwies.

Tabelle 2. Die Anfälligkeit versciedener Apfelunterlagen gegenüber dem Apfelmosaikvirus

Unterlagen mit starker Anfälligkeit		Unterlagen mit mittlerer Anfälligkeit		Unterlagen mit schwacher Anfälligkeit	
EM XXV	14.0	MM 109	5.6	EM IV	1.0
Crab C	12.0	EM IX	4.6	EM II	0.7
EM VII	10.5	EM XI	3.6	MM 111	0.7
MM 104	9.6			EM XVI	0.5

Hervorzuheben ist ferner, dass sowohl Luckwill (1953) als auch Posnette und Cropley (1956) bei der Sorte Miller's Seedling relativ schwere Symptome beobachteten, während in den eigenen Versuchen bei dieser Sorte die geringste Anfälligkeit festgestellt werden konnte.

Die unterschiedliche Anfälligkeit der einzelnen Sorten und Unterlagentypen lässt die Frage berechtigt erscheinen, ob die Ausbreitung des Apfelmosaikvirus bei den einzelnen Sorten variiert und damit auch das systemische Verhalten des Virus sortenabhängig ist.

Zur Klärung dieser Frage wurden im Herbst 1959 von je zwei infizierten Pflanzen aller vorhererwähnten Sorten mit Ausnahme der vegetativen Unterlagentypen je ein Reis aus der Kronenmitte der Pflanze geschnitten und nur die Augen, welche sich oberhalb des letzten symptomtragenden Blattes befanden, der Reihe nach von der Basis bis zur Spitze einzeln auf Grahams-Sämlinge okuliert. In Einzelfällen wurden Augen, die aus den Achseln symptomtragender Blätter stammten, ebenfalls dieser Prüfung unterzogen. Als Kontrollen dienten Reiser von virusfreien Pflanzen der jeweiligen Sorte. Die Auswertung der Versuche erfolgte im Juli 1960, und die Ergebnisse sind in den Tabellen 3 und 4 zusammengefasst.

Während sich bei den Kontrollen in keinem Falle Symptome auf Grahams-Sämling zeigten, trat bei den Sämlingen, welche mit

Augen von infizierten Bäumen veredelt worden waren, ein unterschiedlicher Prozentsatz infizierter Pflanzen auf.

Wie ungleichmässig das Apfelmosaikvirus dabei in einjährigen Trieben einer Pflanze verteilt sein kann, mag Tabelle 3 erläutern.

Tabelle 3. Prüfung des systemischen Charakters des Apfelmosaikvirus bei verschiedenen Apfelsorten

Getestete Sorte Reisnr.		Reihenfolge der Augen von der Basis bis zur Spitze des Triebes, Veredlung auf Grahams-Sämling
Taunton Cross	1	— — — —
»	2	+ — — — —
»	3	+ — — + + +
»	4	+ + + — — — + + — — +
Bramley's Seedling	1	+ + + — + + — +
»	2	+ + — + + — — — + + —
Horneburger Pfannk.	1	⊕ + + + ⊕ ⊖ + + ⊖ + ⊕ + + ⊕ + ⊕ + + + —
»	2	+ +
Finkenwerder Herbstp.	1	+ — + — — + — — + — + + + + + — + — + — + + +
»	2	+ + + — + + + — + + + + + + + + + + + + + + + +
Ingrid Marie	1	+ + + + + +
»	2	+ + + + + + — — + + + + + + + + + — + — — + +
Krüger Dickstiel	1	+ + — + + — — —
»	2	— + + + + + + + + — + — — — — — — — — — — — —
Weisser Klar	1	+ + + + — + — + + + + — + + — + + + + — + + — +
»	2	— — + — + —

· Grahams-Sämling mit Mosaiksymptomen; die zur Veredlung benutzten Augen stammten aus den Aschseln symptomloser Blätter,

⊕ Grahams-Sämling mit Mosaiksymptomen; die zur Veredlung benutzten Augen stammten aus den Aschseln symptomtragender Blätter,

— Grahams-Sämling ohne Mosaiksymptome; die zur Veredlung benutzten Augen stammten aus den Achseln symptomloser Blätter,

⊖ Grahams-Sämling ohne Mosaiksymptome; die zur Veredlung benutzten Augen stammten aus den Achseln symptomtragender Blätter.

Virusinfizierte und gesunde Augen können von der Basis bis zur Spitze eines Triebes abwechselnd vorkommen, gleichgültig, ob sie aus Achseln symptomtragender oder symptomloser Blätter stammen. In vielen Fällen sind jedoch nur die basalen Augen eines Triebes infiziert, während die apikalen Augen sich als virusfrei erweisen (s. Taunton Cross, Krügers Dickstiel), sodass die Möglichkeit zum Neuaufbau von virusfreien Jungpflanzen nicht nur

Tabelle 4. Prüfung des systemischen Charakters des Apfelmosaikvirus bei verschiedenen Apfelsorten

Getestete Sorte	Anzahl mosaikkranker Sämlinge/Gesamtanzahl der Sämlinge				Anzahl mosaikkranker Sämlinge in %			
	Reis a		Reis b		Reis a		Reis b	
Golden Delicious	1/6		1/6		17		17	
Roter Martini	3/4	9/19	8/8	14/20	75	47	100	70
Jonathan	2/2		6/9		100		67	
Taunton Cross	0/4	4/6	1/5	6/11	0	67	20	55
Tydeman's Late Orange	9/17	4/4	4/6	5/13	53	100	67	39
Bramleys's Seedling	6/8		6/11		75		55	
Cox Orange Renette	11/20		5/11		55		46	
Winston	1/3		6/14		33		43	
Rival	8/10		7/9		80		78	
Grahams Jubiläum	0/6		0/9		0		0	
Allington Pepping	4/7		8/8		57		100	
Laxton's Epicure	5/9		—		65		—	
Tydeman's Early Worcester	—		—		—		—	
Worcester Pearmain	5/6		3/8		83		38	
Melba	9/9		3/6		100		50	
Echter Glocken	8/15		5/7		53		71	
Goldparmäne	2/9		1/13		22		8	
Blenheim Orange	3/13		2/8		23		25	
Horneburger Pfannkuchen	4/4		24/24		100		100	
Newton Pepping	17/22		3/20		77		15	
Ellison's Orange	0/6		0/6		0		0	
Finkenwerder Herbstprinz	14/23		21/23		61		91	
Geheimrat Oldenburg	9/14		9/12		64		75	
Coulon Renette	10/11		5/5		91		100	
Brettacher Sämling	24/26		4/10		92		40	
Schöner aus Bath	0/9		1/15		0		7	
Ingrid Marie	6/6		18/23		100		78	
Transparent	5/6		5/7		83		71	
Zabergäurenette	4/17		3/14		24		21	
Gravensteiner	13/18		8/28		72		29	
James Grieve	4/9	4/10	3/4		44	40	75	
Schöner von Boskoop	0/20		4/5		0		80	
Early Victoria	6/20		3/11		30		27	
Krügers Dickstiel	4/11		9/24		37		38	
Jacob Fischer	11/25		5/23		44		22	
Weisser Klarapfel	18/24		3/6		75		50	
Miller's Seedling	0/17		4/17		0		24	

aus apikalen Augen symptomloser Triebe (s. POSNETTE und CROPLEY, 1956, S. 127) sondern auch aus apikalen Augen sichtbar infizierter Triebe besteht (Basisblätter mit deutlichen Symptomen, Spitzenblätter ohne Symptome). Die Methode gibt jedoch keine Sicherheit, da die Verteilung des Virus nicht nur innerhalb eines Triebes, sondern auch innerhalb der gesamten Pflanze verschieden sein kann, wie das Beispiel der Sorte Taunton Cross zeigt, bei der in zwei Fällen apikale Augen virusfrei sind, in zwei anderen jedoch eine Infektion mit dem Apfelmosaikvirus aufweisen. Sehr deutlich zeigt auch die Sorte Horneburger, dass die Verseuchung apikaler Augen sehr stark sein kann, obwohl die Augen aus den Achseln symptomloser Blätter stammten und sämtliche Blätter bei Reis 2 symptomlos waren.

Vergleicht man die Infektionszahlen der anfälligen Sorte Taunton Cross mit der schwach anfälligen Sorte Weisser Klar, so ergibt sich, dass die Ausbreitung des Virus in der schwach anfälligen Sorte nicht geringer zu sein braucht als bei der stark anfälligen. Die in Tabelle 4 angegebenen Daten weisen ebenfalls daraufhin, dass der Prozentsatz viruskranker Augen bei einzelnen Trieben eines Baumes (Taunton Cross, Tydeman's Late Orange, James Grieve u.a.) ebenso stark variiert wie bei den einzelnen Sorten untereinander. Mit Ausnahme der Sorten Taunton Cross (1 Reis), Grahams Jubiläum, Ellison's Orange, Schöner aus Bath, James Grieve und Miller's Seedling tritt stets ein wechselnder Prozentsatz infizierter Augen auf, die mehr oder minder ungleichmässig innerhalb eines Triebes und innerhalb der gesamten Pflanze verteilt sind. Ein unterschiedliches systemisches Verhalten des Apfelmosaikvirus bei verschiedenen Apfelsorten in Abhängigkeit vom Toleranzgrad der Sorten lässt sich aus diesen Versuchen nicht ableiten.

SUMMARY

In Western Germany apple mosaic occurs on numerous apple varieties.

Thirty-eight different apple varieties and eleven root-stocks were infected with a single strain of apple mosaic virus by chip-budding and the symptom expression on these different varieties and stocks was then observed over a period of three years. There were considerable differences in the susceptibility within the different varieties and stocks, the greatest susceptibility being found in Golden Delicious,

Roter Martini, Jonathan and Taunton Cross, the lowest in Miller's Seedling, Weisser Klarapfel, Jacob Fischer and Krügers Dickstiel. When comparing the stocks, the most susceptible stocks were EM XXV and Crab C, while EM 111 and EM XVI were the least susceptible ones.

Buds taken from axils of symptomless leaves showed infection with apple mosaic virus in a variable degree, and the distribution of apple mosaic virus in the plant was irregular.

DISCUSSION

Luckwill: Is it possible that Miller's Seedling was affected by a mild strain of apple mosaic virus before being infected by the Jonathan mosaic strain?

Hamdorf: Indexed on Graham's Seedling, (Graham's Seedling sometimes seems to be a better indicator than Lord Lambourne), Miller's Seedling proved to be free from apple mosaic virus.

Millikan: How many leaves of a young shoot did not show symptoms?

Hamdorf: The number of leaves showing no symptoms varied among the varieties and also among the different shoots of the same tree from 2 to 28.

Baumann: The apple mosaic virus also is not fully systemic in branches which are two and more years old. This fact should be considered on indexing mother-trees.

Hamdorf: Experiments about the systemic character of apple mosaic virus, carried out in 1958, verify this opinion.

Schuch: Do you think that symptoms can appear next year where symptoms have not yet been observed?

Hamdorf: It may be. Generally symptoms appear in the first year after inoculation, but in a few cases (observations from nurseries) symptoms can be observed in the second one.

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Chlorotic Leafspot and Its Relation to Other Virus Disorders of Apple

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Recent investigations have established the presence of one or more latent viruses in orchard trees and vegetatively propagated apple rootstocks. One of these, named the stem pitting virus (SPV), is detected by grafting infected tissue on a Virginia Crab tree free from the virus (2). Another has been briefly described (6, 7) and named chlorotic leaf spot virus (CLSV). It is detected by grafting infected tissue on a Russian apple, R12740-7A. The purpose of this study was to establish the relation of chlorotic leaf spot with stem pitting and certain other transmissible diseases of apple and to describe the chlorotic leaf spot disease more fully.

Materials and Methods

The apple varieties used as indicators were R12740-7A (7); Virginia Crab K6 (7); Lord Lambourne P.I. 123747; Spy 227 (from Dr. R. M. Gilmer, New York); *Malus platycarpa* (from Dr. L. C. Luckwill, England) (4); and *M. sieboldii* 2972-22 (11). Trees of the apple varieties indexed were in commercial plantings in Indiana or in experimental orchards of Purdue University. Stools of the East Malling and Malling-Merton rootstocks were maintained by the Doud Nursery, Roann, Indiana.

The indexing was done with nursery trees in the field or with potted plants in the greenhouse. For most of the field trials, the indexing was done by placing one or two buds from the indicator variety into one-year-old trees of the variety to be indexed. During the winter, the trees were pruned to the indicator bud and growth was forced in the spring. All inoculations of Virginia Crab and

all field trials with R12740-7A were carried out in this manner in August, 1956. The field inoculations of Lord Lambourne for the rubbery wood virus (9) were made by placing a bud from the tree to be indexed into one-year-old Lord Lambourne trees in August, 1957. Final observations of all trees in the field trials were made in August, 1960.

In the greenhouse, trees of the indicator varieties were prepared by inserting a bud into a forced one-year-old seedling, in a 5-inch pot. After two weeks, the seedling was cut back to the inserted bud and growth was forced. At the first evidence of growth of the indicator bud, the tree was inoculated by placing one or two buds from a dormant scion of the tree to be indexed into the seedling stem directly below the indicator bud. When the indicator shoots ceased growing, usually 6-8 weeks after inoculation, all inoculated trees were examined for the inner-bark necrosis symptom. Trees showing no foliage or bark symptoms were cut back and another flush of growth was forced.

The greenhouse trials were conducted from January through May in 1959 and in 1960. Minimum night temperatures were maintained at 70°F., but daytime temperatures in 1959 were controlled only by greenhouse vents and often rose into the 80's and 90's. In 1960, better daytime control was obtained by the use of exhaust fans drawing air into the greenhouse through water-soaked excelsior pads.

Results

Description of Chlorotic Leaf Spot Disease on R12740-7A. The first evidence of infection in the greenhouse usually appeared within 21 days after inoculation, depending upon incubation temperature, source of inoculum and other factors not yet defined. Growing points and newly expanding leaves appeared flaccid about 24 hours prior to the appearance of first leaf symptoms. The shoot tips then regained turgidity and small circular chlorotic spots appeared in the young leaves, usually in one half of the youngest leaf. The clorotic spots increased in number and were accompanied by irregular necrotic areas. As growth of the shoot continued, the affected leaf halves failed to develop normally,

resulting in a marked unilateral distortion. Usually growth of the entire shoot ceased shortly after leaf symptoms appeared. Trees inoculated in the field in the fall showed symptoms shortly after new growth appeared in the spring. Affected leaves showed numerous chlorotic spots but little necrosis and in general were not markedly distorted. In severe cases, however, leaves never attained full size; they were severely deformed and shoot growth was reduced to rosettes.

Associated with the leaf symptoms, both in the greenhouse and the field, was a necrotic flecking of the inner bark and cambial region. These flecks appeared 15 to 20 days after first leaf symptoms and were irregular to elliptical in shape. In severe cases, they coalesced to form streaks. In the field, necrotic areas

Table 1. Incidence of chlorotic leafspot virus in trees of apple scion varieties and stools of apple rootstocks in Indiana

	No. Infected[1,2] No. Indexed	Percent of Clones Infected
Orchard Trees		
Delicious	27/39	69
Golden Delicious	12/16	75
Jonathan	21/23	91
Rome	39/44	90
Steyman Winesap	6/10	60
Turley	25/31	81
Rootstock Stools		
EM II	23/26	88
EM VII	27/28	96
EM IX	27/27	100
MM 104	0/20	0
MM 106	2/20	10
MM 109	16/20	80
MM 111	0/20	0

1. The results are recorded as a fraction. The denominator is the number of individual trees or stools that were indexed and the numerator is the number infected.
2. Results are based on 4 indexing trials on R12740-7A in the greenhouse during the spring months of 1959 and 1960, except the Rome and Turley varieties which were indexed on nursery trees in the field.

were less conspicuous during hot weather. Late in the first season and during the second year, the necrotic flecks appeared grouped into elliptical areas of variable size. The woody cylinder below these flecks developed definite pits similar to those on Virginia Crab trees infected with the stem pitting virus (12). In some cases, the main stems near the ground line of chlorotic leaf spot infected trees of R12740-7A were severely pitted.

Infected R12740-7A trees were generally weak and dwarfed. Although many survived four growing seasons, others died, possibly due to increased susceptibility to winter injury.

Incidence of Chlorotic Leaf Spot Virus. Dormant scions were collected at random from trees of a number of apple varieties in Indiana orchards during the early winter of 1959 and of 1960. Scions were also collected at random from a number of stools of East Malling and Malling-Merton rootstocks. The incidence of the CLSV in each of these varieties was determined by greenhouse indexing of R12740-7A trees (Table 1). The virus was widespread in the orchard trees and in the East Malling rootstocks tested. The incidence was also high in the MM 109 rootstock but low in MM 106. The virus was absent in the stools tested of MM 104 and MM 111. No stools of EM IX and only one of the EM VII stools were free from the virus. All apparently virus-free stools of all the rootstocks are being reindexed on R12740-7A and are being indexed for the first time on the other indicator varieties. A great deal of variability occurred in the first greenhouse

Table 2. Variability in chlorotic leaf spot virus detection in greenhouse indexing trials with R12740-7A indicator trees

	No. Stools Indexed	No. Infected[1] / No. Indexed				
		Trial 1[2]	Trial 2	Trial 3	Trial 4	Total
EM II	26	3/26	10/23	7/13	3/6	23/26
EM VII	28	8/28	12/20	5/8	2/3	27/28
EM IX	27	1/27	16/26	9/10	1/1	27/27

1. See footnote 1, Table 1.
2. Trial 1 made in late spring 1959; Trials 2, 3 and 4 made in early spring, 1960. After Trial 1 only negatively reacting stools were indexed in succeeding trials.

Table 3. Effects of temperature during incubation on chlorotic leafspot symptom development on R12740-7A indicator trees inoculated at budbreak

Temperatures °F.		No. Infected[2,3]
Light Period	Dark Period[1]	No. Indexed
60	60	5/15
70	60	2/8
80	60	2/7
70	70	12/14
80	70	2/7
80	80	4/14
90	90	2/7

1. The thermoperiods were 8 hours (during the light period) and 16 hours. The photoperiods were 12 hours light and 12 hours dark. The temperatures indicated for the dark period also prevailed for 3 hours following and 1 hour preceding the dark period.
2. See footnote 1, Table 1.
3. Source of infected buds used in the inoculation was C-13 Virginia Crab.

indexing test of the English rootstocks (Table 2). The first indexing trial was conducted during a warm period in April and May of 1959 when greenhouse daytime temperatures rose frequently to above 90°F. More positive cases were indicated in the second trial (February, 1960), when temperatures were better controlled, and still more in the third and fourth trials. Since temperature effects on symptom expression in the R12740-7A variety were suspected, an experiment was carried out in the springs of 1959 and 1960 in the controlled climate laboratory. Since the results of the two experiments were similar, the data were combined in Table 3. Under the conditions of these experiments, the most favorable temperature combination for symptom expression was a constant 70°F.

Relation Between the Chlorotic Leaf Spot and the Virginia Crab Stem Pitting Diseases. The observed pitting and bark necrosis in chlorotic leaf spot infected R12740-7A trees raised the question of the relationship between these two virus diseases. Orchard trees that had been examined for the stem pitting symptom were indexed in the field on young trees of Virginia Crab K6 and R12740-7A. Symptom developments were observed for four grow-

Table 4. Association of the leaf spot and inner-bark necrosis symptoms induced by chlorotic leaf spot virus in R12740-7A trees with the stem pitting symptom induced by the stem pitting virus in Virginia Crab trees

Clone	Variety/Rootstock	Amount of Pitting at Base of Orchard Trees	Symptoms on Inoculated Indicator Varieties		
			Virginia Crab Stem Pitting	R12740-7A Leaf Spotting & distortion	R12740-7A Inner-Bark Necrosis
C–13	Virginia Crab/(Self-rooted) . .	Severe	+	+	+
C–7	Grimes Golden/Virginia Grab	Severe	+	+	+
C–10	Golden Delicious/Virginia Crab.	Severe	+	+	+
C–14	Blackjon/Virginia Crab	Severe	+	+	+
C–8	Virginia Crab/(Self-rooted) . .	None	0	+	+
C–11	Richard/Virginia Crab.	None	0	+	+
C–12	Starking/Virginia Crab	None	0	+	+
C–2	Winesap/Seedling.	None	0	+	+
C–101	Early Victoria/Seedling	None	+	0	0
C–103	Unknown/Unknown	Unknown	+	0	0
C–6	Grimes Golden/Virginia Crab	Light	0	0	+
C–9	Virginia Crab/(Self-rooted) . . .	None	0	0	0
C–23	Hyslop Crab/Seedling	None	0	0	0
C–91	Antonovka Shafran/Seedling	None	0	0	0

ing seasons (Table 4). Most trees with severely pitted Virginia Crab stem pieces transmitted both the SPV and the CLSV. Three trees with non-pitted Virginia Crab stems did not transmit the SPV but all three transmitted the CLSV. Two trees transmitted the SPV, but not the CLSV. One of these was on a seedling rootstock and did not show pitting at the base of the tree, but the condition of the second is not known. The inner-bark necrosis symptom in the R12740-7A indicator was associated with the leaf spotting symptom in every case except one (C-6 clone). It is the only exception encountered among the several hundred orchard trees indexed on R12740-7A during the past four years, and is being further studied.

Relation Between the Chlorotic Leaf Spot Disease in R12740-7A

and Other Apple Virus Diseases. Lord Lambourne P.I. 123747 produces young trees normal in growth and appearance when propagated on seedling rootstocks. This clone was indexed on R12740-7A and found to contain the CLSV. Sixty-nine trees from commercial orchards or experimental plantings were indexed on Lord Lambourne and two of these induced positive rubbery wood symptoms in the indicator trees. Consequently, we may conclude that the rubbery wood virus is distinct from the CLSV or is a complex with the CLSV as a component.

A number of isolates of apple mosaic and other apple viruses previously described were indexed comparatively on R12740-7A, Spy 227, *Malus platycarpa* (Luckwill) and *M. sieboldii* 2972-22. The apple mosaic isolates were those used previously (7). With few exceptions, isolates that induced chlorotic leaf spot in R12740-7A induced leaf symptoms in the other three indicator hosts. The CLSV was present in seven of the eight isolates of apple mosaic and in single isolates of each of the following virus diseases: dwarf fruit and decline (1), apple green mottle (8), Spy 227 lethal (3), and scar skin (5).

DISCUSSION

Our data lead to the conclusion that the CLSV indicated by R12740-7A, and the SPV indicated by Virginia Crab are distinct latent viruses that may occur separately or together in trees of commercial apple orchards in Indiana. The CLSV appears to be distinct from the rubbery wood virus and it occurs widely among the older apple varieties. Since most of these varieties have been propagated on seedling rootstocks for an indefinite period in the past, the high incidence of the CLSV suggests the possibility of orchard spread. Data are not yet complete on the incidence of the SPV as a latent virus in Indiana orchard trees propagated on seedling rootstocks.

It is not known how deleterious infection by the CLSV is to growth and production of commercial orchard trees on seedling rootstocks, for such trees appear to have little sensitivity to the virus. Several *Malus* species are sensitive, however, and the virus undoubtedly plays a role in propagation failure (10).

Among the rootstocks studied, East Malling II, VII, and IX and Malling-Merton 106 and 109 are contaminated with the CLSV. Malling-Merton 104 and 111 are free from CLSV. The variable results among indexing trials (Table 2) suggest that not all buds of a dormant scion are infectious. Trials are underway with EM VII to test the possibility of obtaining CLSV-free buds from an infected scion.

The contamination of isolates of apple virus diseases with the CLSV is widespread and significant. Seven of eight isolates of apple mosaic from widely scattered locations were contaminated with the CLSV. Special precautions to determine the presence of the latent CLSV in experimental apple materials are necessary. Allowance should be made also for the sensitivity of the experimental plants to the chlorotic leaf spot virus.

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Latent Viruses in Apple Rootstock and Scion Varities

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Apples, compared with stone fruits, or even with pears, are relatively tolerant of virus infection. Of the dozen or so viruses which have been recorded on apple, the majority produce symptoms on only a few varieties. Examples are chat fruit of Lord Lambourne, stem-pitting of Virginia Crab, rubbery wood which affects Lord Lambourne, James Grieve, Golden Delicious and a few other varieties, and flat limb such as develops on Gravenstein. Even apple mosaic, economically the most important apple virus, produces symptoms on only about 50 per cent of commercial varieties. Yet we know from indexing experiments that many of these viruses are widely distributed in latent form, both in scion varieties and in certain clonally propagated rootstocks. Just how wide-

Table 1. Latent viruses in cider-scion nursery. Long Ashton indexed on Lord Lambourne

	Tested	Rubbery wood	Mosaic	Chat fruit
No. of varieties	53	35	13	11
No. af trees	352	116	43	15

spread these viruses are can be seen from Table 1, which shows the result of indexing our cider variety scion-nursery at Long Ashton. All these trees appear healthy, but as can be seen from the table a very large percentage of them are carrying latent virus.

More recently we have discovered another group of viruses which occur in latent form in apples and which, as far as is known, produce no symptoms of disease on any commercial

variety. We recognise these viruses by the symptoms they produce on *Malus platycarpa*. Three different types of symptoms occur on *M. platycarpa* after inoculation from commercial scion varieties and rootstocks. These symptoms may occur separately, or they may occur together in all possible combinations, so that we are satisfied that they represent three distinct viruses.

Line pattern: In infected trees the leaves are smaller than normal and are often somewhat distorted and cupped in shape. They show an irregular diffuse chlorotic line pattern with a tendency to form small but distinct ring spots. Severely infected leaves are often shed prematurely.

Dwarfing: Normally *M. platycarpa* on a seedling stock will attain a height of approximately 1 metre during its maiden year. Infected trees show varying degrees of dwarfing, maiden growth ranging from 30 to 60 cm. The dwarfing persists during the second and third years and probably throughout the life of the tree.

Scaly bark: The bark of one-year old shoots takes on a roughened, scaly appearance often accompanied by a slight swelling. These symptoms may occur on the main stem, but often they are particularly prominent near the bases of one-year old lateral shoots. Frequently the shoot is completely girdled and, where infection is severe, the whole tree may be killed. Secondary infection of the bark lesions by fungi commonly occurs.

Table 2. Latent viruses in apples. Indexed on *Malus platycarpa*

	No. of varieties	Line pattern	Scaly bark	Dwarf
Cider apples.....	21	20	17	13
Dessert and culinary .	17	15	15	12

The most remarkable thing about these *platycarpa* latent viruses is their very widespread distribution. Table 2 shows the results of indexing 21 varieties of cider apple and 17 varieties of dessert and culinary apple at Long Ashton. In this experiment two trees of each variety were indexed. Nearly all varieties prove to be carrying latent line pattern and a very high proportion are also carrying scaly bark and dwarfing.

We have also found that many of the Malling and some of the Merton-Malling clonal rootstocks are carrying these latent viruses. Results of indexing trials with these clonal stocks are shown in Table 3. Since this experiment was completed we have also found

Table 3. Latent viruses in apple rootstocks. Indexed on *M. platycarpa*

Rootstock	Line pattern	Scaly bark	Dwarf
M. I	X	X	X
M. II	X	X	-
M. IV	X	X	X
M. VII	X	X	X
M. IX	X	X	X
M. XII	-	-	-
M. XVI	-	-	-
M. XXV	X	X	X
M. XXVI	-	-	-
MM. 104	-	-	-
MM. 106[1]	-	-	-
MM. 109	X	X	X
MM. 111	-	—	-
Crab C[2])	X	—	X

1. 1 clone infected with line pattern and dwarf.
2. Some clones free of line pattern or dwarf.

latent line pattern in certain stools of M.XVI. It is interesting to note that most of the newer series of Merton-Malling stocks, with the exception of MM. 109 and certain clones of MM. 106 appear to be virus-free, as is also the relatively new M. 26.

These *platycarpa* latent viruses are distinct from rubbery wood, chat fruit and mosaic viruses. They also appear to be distinct from stem-pitting virus, though we have not yet investigated their relationship with chlorotic leaf spot described by Dr. Smay. In spite of their wide distribution, these viruses, as far as we know, have no vectors other than man himself. Like other apple viruses they appear not to be seed transmissable. Seedling apples which have been growing in the field for 10 years, as part of a

breeding project, have not yet become infected with these viruses. We have also tracked down the original seedling tree of the variety Bramley's Seedling, which is still growing in a garden in Southall, England, and which is now over 100 years of age. Buds from this tree indexed on to *M. platycarpa* produced no symptoms: by contrast, all the samples of Bramley's Seedling from commercial orchards that we have indexed are carrying all three viruses.

From the theoretical point of view these latent viruses are of considerable interest, but from the practical point of view the important question is – what effect if any, do these latent infections have on the performance of the tree – not only its vigour of growth and cropping – but also possibly on its susceptibility to diseases, on the uptake of nutrients, on its reaction to spray chemicals and on stock/scion compatibility. All these aspects of tree performance could conceivably be affected by the presence of latent virus. This is a matter on which we urgently need information, for what information we have for apples and for other fruits, points to the fact that viruses which are apparently latent, in that they produce no obviuos disease symptoms, do in fact effect vigour and productivity. For example, we have recently found at Long Ashton that infection of M. I stocks with latent rubbery wood virus significantly reduces the productivity of the stoolbed over a period of four years measured as the number of stocks produced annually from each stool.

Before we can set up field experiments to determine the effect of these latent viruses on the performance of the tree in the orchard it is necessary to obtain virus-free trees to act as controls. The wide distribution of the *platycarpa* viruses makes this difficult. There are however two possibilities. Firstly, we can use new seedling varieties which have not yet become infected, and propagate them by budding on to seedling stocks or on to clean stocks of the MM series. Secondly, it might prove possible to eliminate these viruses from infected scion material by heat therapy treatment followed by tip propagation. We are at present investigating both these possible approaches at Long Ashton. We have also tried to speed up our indexing work by the transmission of these viruses to herbaceous host plants, but so far without success.

DISCUSSION

Posnette: Dr Luckwill gave evidence that the latent viruses do not spread except by grafting. How does he suggest that plum and pear trees become infected? The presence of healthy and infected trees in the MM clones also suggests spread in the field.

Luckwill: Cross inoculation between apple, pear and plum could have occurred by natural root grafting in mixed orchards. If natural spread occurs in the field it is difficult to explain why seedling apples do not become infected, even after long periods of time.

The Stem Pitting and Scar Skin Disorders in Apple

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Stem Pitting

Abstract

The stem pitting virus (SP) appears to be widespread in most American varieties of apple. Ordinarily, it seems to be a latent virus of little economic importance unless scions from a diseased source are budded or grafted on a sensitive body or rootstock such as Virginia Crab or Spy 227. This trouble was first noted when our growers started using Virginia Crab as a hardy understock

Fig. 1. Close up of Virginia Crab Stem Piece Top-worked with Diseased Golden Delicious

Fig. 2. Close-up of Virginia Crab Stem Piece Top-worked with healthy Golden Delicious

following the severe late freeze of November 11, 1940. Since that time this stock has grown into disfavour due to its sensitivity to infection with this virus as well as the extreme vigorousness if disease-free.

Symptoms of the Disease

Initially symptoms appear as small boat-shaped indentations on the wood beneath the cambium. Gradually these pits enlarge and develop into cracks in the bark which may be one-half inch wide and several inches long. The pitting becomes more severe as the tree matures causing a pronounced dwarfing effect. Scion rooting may be affected since the Virginia Crab worked with diseased scions generally fail to root and are dependent upon the seedling nurse root. The virus appears to stimulate sucker growth from the seedling root since affected trees usually show this characteristic.

Transmission Studies

Symptoms of this disease were experimentally transmitted in 1956 (1). In the previous two seasons scions from water sprouts of Virginia Crab developing from a stem piece top-worked with

Fig. 3. Virginia Crab buddings as indicators for SP. Trees on left budded with healthy Golden Delicious, center unbudded control, trees on right budded with pitted Golden Delicious

healthy Golden Delicious were bench grafted on seedling apple stocks. Trees from this material were used for the transmission tests and form source material for subsequent studies.

In 1954 and 1955 successful transmission of SP was accomplished as indicated in Fig. 3. In every instance where whips of healthy Virginia Crab were inoculated with buds from varieties growing on pitted Virginia Crab stem pieces, typical symptoms were noted the second year. Virginia Crab whips inoculated with buds from healthy sources remained symptomless. Since that time healthy Virginia Crab has been used extensively as an indicator.

Natural spread is still unknown but appears to be quite rapid under some conditions.

Histological Studies

The Maine workers (2) have studied the morphological changes that occur in the phloem, xylem and cambium of Virginia Crab as a result of infection. Stained slides from diseased material show extensive sieve tube degeneration, pronounced distortion in the cambial zone at the end of a wood ray and often multinucleate nuclei in the phloem mother cells.

HOST RANGE

The virus probably will infect many species or clones in the pomaceous group but presently our evidence is confused by the presence of latent viruses in many of our cultures. (See papers of Shay and Luckwill). Striking foliar symptoms have been observed on *Prunus tomentosa* seedlings and clones of *Malus floribunda, Amelanchier spp., Crataegus mollis,* and *C. crus-galli.*

ECONOMIC IMPORTANCE

Wood pitting of Virginia Crab trunks top-worked to commercial varieties appears to be widespread in many fruit growing sections of the United States and Canada. In many cases, the resulting double worked trees are dwarfed only slightly and fruit production is exellent, both in quality and quantity. Most varieties on seedling roots appear to be little affected, if any, with the disease and grow quite normally.

CONTROL MEASURES

The use of seedling or resistant clonal rootstocks appears to be the logical method of controlling this disease. Nurseries are initiating programs to produce disease-free nursery trees.

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Scar Skin

ABSTRACT

The scar skin disorder first was noted in 1955 when severely blemished fruits of the Red Delicious apple were sent into the Station for identification. A survey in the affected apple producing areas indicated that this disease was present in several additional orchards. Since then it has been found in one other apple producing area of the state and symptoms similar to this have been reported in other states. All fruits on affected Red Delicious trees show the characteristic symptoms of the disease and are worthless for commercial or culinary use.

SYMPTOMS OF THE DISEASE

Essentially the symptoms on Red Delicious are restricted to the fruit, but are so spectacular that the entire crop of the affected tree is worthless. Diseased trees tend to have somewhat sparcer foliage and seem to stimulate a little more anthocyanin production in the stem piece of young trees. In early June, the affected fruits show a water-soaked blotching on the blossom end. Similar but smaller blemishes soon appear on the sides of the fruit radiating

Fig. 4. Early June Symptoms of Scar Skin on Red Delicious (left). Healthy fruit on right

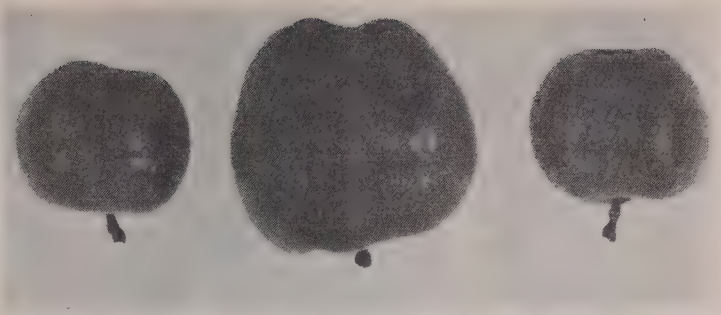

Fig. 5. Early August Symptoms of Scar Skin on Red Delicious (left & right). Healthy fruit in center

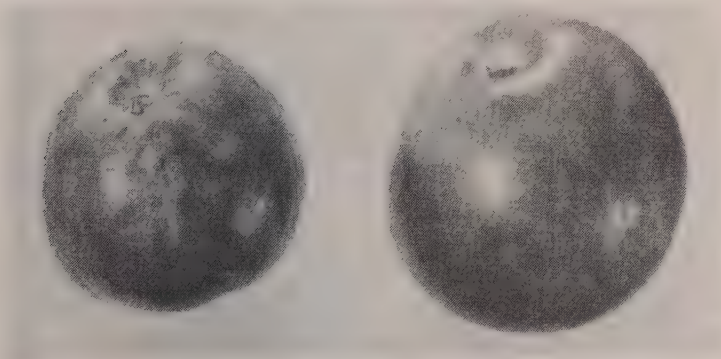

Fig. 6. Harvest Symptoms of Scar Skin on Red Delicious (left). Healthy fruit on right

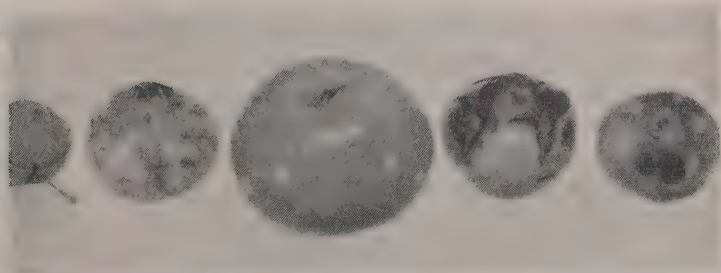

Fig. 7. Symptoms of Scar skin on Stark's Earliest. Worked on diseased Red Delicious (right and left). Healthy Stark's Earliest in center

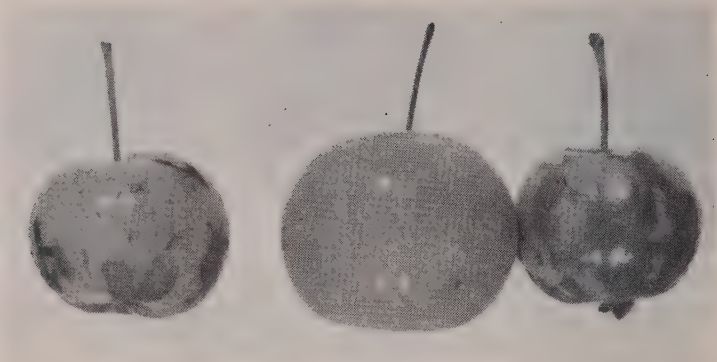

Fig. 8. Symptoms of Scar Skin on Virginia Crab from fruiting stem piece bearing Scar Skin on Red Delicious. Healthy fruit in center

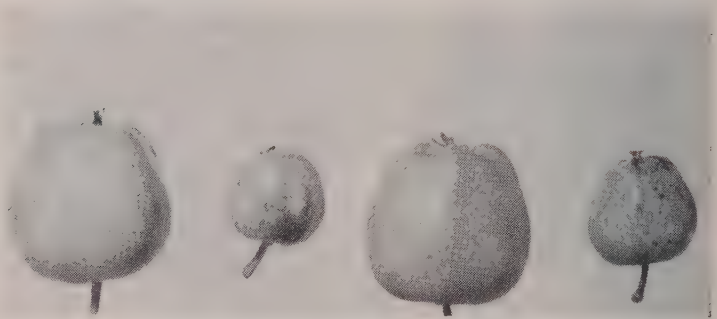

Fig. 9. Scar Skin on Red Delicious. Fruit on left from diseased tree. Fruit 3rd from left from scion of Red Delicious bearing the fruit shown on 2nd from left. Healthy fruit on right

from the stem end. By late June scar tissue develops in the epidermis, especially around the calyx. By early July when the fruits are a little over one inch in diameter, irregular patches of epidermal scar tissue develop on the sides of the fruits. At harvest time as much as 50 per cent of the epidermal tissue may develop this scar skin replacing much of the natural pigmentation.

TRANSMISSION STUDIES

Limited transmission studies along with convincing field observations indicate that a transmissible entity is involved. Disease-

free scions of Red Delicious and Stark's Earliest worked on infected trees produced severely blemished fruits. The constant association of severely scarred Virginia Crab fruits on the fruiting body stocks bearing infected Red Delicious apples suggest that this variety may be a good indicator.

VARIETAL HOST RANGE

In addition to the Red Delicious, several other varieties are known to show symptoms of this disease although only Stark's Earliest approaches Red Delicious in the severity of symptoms. Susceptible varieties include Jonathan, Red Gold, Turley, Staymen and Rome Beauty insofar as marked reduction in flavour and size are concerned. Lodi, Golden Delicious and Minjon appear to be tolerant but are somewhat reduced in size and mildly off-flavour.

ECONOMIC IMPORTANCE

Fruits on affected trees of Red Delicious never ripen but tend to remain starchy even under the most favourable ripening conditions. Quality is adversely affected since the diseased fruits possess a merked off-flavour. In the case of Red Delicious this off-flavour is so marked that the fruits are worthless even for juice. On other varieties tested thus far the main characteristics are a less pronounced off-flavour accompanied by size reduction in the fruit. This off-flavour has not been chemically identified but tests indicate that there are no significant differences in the acid or total solids fractions.

CONTROL MEASURES

Prompt removal of affected trees is recommended if the production of the Red Delicious is desired. Top-working diseased trees with a resistant or tolerant variety are also suggested.

DISCUSSION

Cropley: We find a fruit symptom on Virginia Crab (small fruits, ribbed at the calyx end) associated with stem pitting. Do the American workers find such an association.

Millikan: Yes, but not so marked as the fruit symptoms I saw in England. In America we see the fruit symptom early in the season, but it becomes less marked as the season progresses.

Apple proliferation disease

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At the request of several members of the fourth symposium on virus diseases of fruit trees in Europe held at Lyngby in July 1960, a seminar was organized to discuss the apple proliferation disease. The author first summarized the most important points about this disease and showed coloured slides of symptoms. This paper reviews the information available at that moment about the disease.

Apple proliferation disease, also called apple witches' broom, is widespread in several countries of Europe. It has been reported from Holland (15, 16), Denmark (12), Finland (1), Germany (4, 13), Switzerland (3, 5, 6, 7), Austria (9), Czekoslovakia (2), Italy (8, 10, 11, 17-21) and France (14), and probably exists in other European countries. It is one of the most important virus

Fig. 1: Small rosette of leaves developing in autumn from a terminal bud of Golden Delicious infected by apple witches' broom virus. Changins, octoLer 1960

diseases of apple trees from an economical point of view, because it can affect the size and quality of the fruit and cause a decline in the growth of the trees.

So far, apple witches' broom virus has been transmitted between apple varieties and from apple to pear trees (18, 19). Boskoop, Gravenstein, Starking, Golden Delicious and Winterbanana appear to be especially sensitive to this virus. No tolerant or resistant variety has yet been found. Rootstocks and seedlings are also sensitive.

Fig. 2: Autumnal growth of two terminal buds on infected Lord Lambourne. Changins, october 1960

Description of symptoms

The most important features of this disease are described here from the author's observations and experiments in Switzerland (3, 6, 7).

One of the first noticeable symptoms is usually an abnormal growth of the terminal buds in autumn. Instead of the dormant terminal bud that appears on a normal shoot, a rosette of young leaves develops late in the season and is often infected by powdery mildew. Flower buds often open in autumn. Small fruits may even appear in November. Some of these flowers are abnormal, with phylloid characters.

The most typical symptom of the disease, if not the most frequent, is the premature development of axillary buds, which grow instead of remaining dormant until the next spring, and develop into the so-called "witches' brooms". These abnormalities usually

Fig. 3: Healthy shoot (left) and "witches' broom" (right) on Golden delicious. Changins, september 1959

appear at the apex of the vigorous shoots, especially on suckers. They are often infected by powdery mildew. One important feature of the "witches' broom", is the small angle the secondary twigs form with the main shoot.

The leaves of infected trees, particularly those of secondary twigs, are irregularly dentate and smaller than normal ones. The

Fig. 4: Leaf of infected tree (left) and healthy leaf (right) of the variety Gravenstein

stipulae are often very long, whereas the petioles are rather short. Infected trees or parts of trees leaf earlier and flower somewhat later than healthy ones. In Switzerland, early reddening or bronzing of the leaves is usually associated with witches' broom virus infection, together with a certain degree of chlorosis. It is likely that many cases of chlorosis commonly attributed to an excess of lime are really due to an infection by this virus.

The fruit of infected trees is smaller than that of healthy trees, whereas peduncles are longer and thinner. The reduction in mean weight of the fruits can be as great as 60 per cent in comparison with fruit of comparable normal trees, whereas the total

Fig. 5: Fruit of infected Golden Delicious and fruit of a comparable healthy tree

loss in yield may reach 95 to 100 per cent. Even when the loss is less severe in weight, the market value of the fruit is practically nil because of its small size. Moreover, it has a rather bad taste, less sugar and less acidity than normal fruit (7). The seeds also are smaller.

Infected trees lack vigour. The shoots are thin and slender. Their bark usually has a reddish brown colour, typical of a poor growth. There is often an early defoliation. Necroses appear in the bark, and some of the branches may dry up. In some cases, the entire tree dies. However, when the disease is less severe, the tree recovers after the shock symptoms of the first two or three years. After several years, it may even give normal fruit again.

The effect of the virus on the tree seems to depend to a large extent on the quality of the soil. Trees in good soil, with enough fertilizer, are less affected than trees growing in poor soil conditions.

High sensitivity to powdery mildew is an important characteristic of the proliferation disease, and the presence of this fungus can be helpful for making the diagnosis. Several phytopatholo-

gists considered the witches' brooms on apple trees as a consequence of powdery mildew infection (11). In fact, witches' broom formation and powdery mildew sensitivity are probably both consequences of apple witches' broom virus infection (3, 6, 8).

Transmission and spread

So far, apple witches' broom virus has only been transmitted by grafting. There is no doubt that the disease spreads in the orchards, but nothing is known about the vector. In some cases, a slow spread to adjacent trees has suggested a soil transmission, but so far this hypothesis has not been confirmed.

The disease is also spread by the use of infected rootstocks and bud- or graftwood. Nearly symptomless trees may yield a high proportion of infected budsticks.

Control measures

Care should be taken to avoid using infected rootstocks and budwood in nurseries, and new plantings should be made only with healthy trees. Any scion with symptoms of proliferation disease should be discarded.

In old plantings, badly affected trees should be rogued in order to avoid the spread of the disease. If the proportion of trees with symptoms is so high that roguing is not worth while, it is advised to give a heavy dressing and take care to maintain the soil in good condition. This will not cure the infected trees, but improve their state of health to a certain extent.

As very little is known about the natural transmission and spread of the disease, it is presently difficult to suggest any further control measure.

DISCUSSION

MORVAN: Showed a few coloured slides about apple proliferation in France, where this disease is widespread. Symptoms are quite similar to those described by Bovey.

Luckwill: The fruit symptoms of "chat fruit" are very similar to those described by Dr. Bovey for the fruits of varieties infected by proliferation virus. There are, however, two important differences:

1) The symptoms of "chat fruit" are, as far as we know, exhibited only by the variety Lambourne. The virus, however, is widely distributed in latent form in other varieties.
2) Unlike proliferation virus, there are no vegetative symptoms associated with "chat fruit".

One of the characteristics of "chat fruit" is that the fruits appear normal until they are about half-grown, when they suddenly cease growth. I would like to ask Dr. Bovey if he observes a similar situation with proliferation virus.

Bovey: No phenomenon similar to that described by Dr. Luckwill has been observed on the fruits of apple trees infected by proliferation virus. The rate of growth of the fruits of infected trees seems to be inhibited during the entire period of fruit formation.

Moreover, it should be noted that this virus produces typical vegetative symptoms on the variety Lord Lambourne and makes it very sensitive to powdery mildew.

Millikan: Is the marked reduction in quality as indicated in lower sugar content of fruit true with several varieties?

Bovey: It has been observed on Golden Delicious, Cox's Orange and Jonathan.

Baumann: I should like to ask Dr. Bovey whether in transmission tests the infection rate is high or low? Do the symptoms in general appear the first year after budding?

Bovey: The virus is easy to transmit by chip-bud inoculation, or by ordinary budding or grafting. The rate of transmission is high. The first symptoms generally appear the year after budding, on the inoculated branches. When infected trees are topgrafted with healthy grafts, the transmission occurs during the first year of growth.

Hansen: Has Dr. Bovey investigated the possibility of cicadellid-transmission?

Bovey: Not yet on a sufficient scale.

Hansen: When Dr. Bovey mentions possible soil-transmission, does it means something like natural root-graft, or transmission from contaminated soil?

Bovey: As already mentioned, some observations suggest a transmission from contaminated soil, but this has not been proved experimentally. It is very unlikely that root-graft could account for the high rate of transmission and spread of the disease in some orchards.

On the other hand, the symptoms of proliferation diseases recall those of leafhopper-borne viruses.

Schuch: In South Germany, the proliferation disease is widespread in large new plantings, which are four, five or six years old, and where

the soil condition is good. Dr. Bovey has said that a recovery of the infected trees is possible. Our growers ask us: "Shall we remove the diseased trees or not?" What is the opinion of Dr. Bovey?

Bovey: It is advisable to remove diseased trees when their proportion is not too high, when the symptoms are very severe or when the disease appears on very young trees. However, no general rule can be given.

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Virus diseases of the Apple tree in the SSR of Latvia

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It has been observed in the SSR of Latvia for some time past that virus diseases, about which relatively little is known so far, considerably reduce the harvest of orchards and vegetable gardens.

Entomologist I. I. Zirnitis states that virus diseases of apple trees in Latvia were noticed 26 years ago. The mosaic disease was observed for the first time on a 20-year old apple tree of the Paradise variety with yellow fruit, in the district of Cesis in 1930. Mosaic was distributed all over the tree which was stunted in growth. The leaves were relatively small and pale-green with a great number of yellow-green spots on the whole leaf surface. The apple tree was observed until the winter of 1939/40, when the orchard was destroyed by frost.

In 1937 some grafts were taken from the diseased apple tree and grafted on five two-year old seedlings. The grafting was successful and in the first two or three years the young trees were healthy and gave normal fruit. However, during the following three years the mosaic disease showed all its symptoms on the young trees. Only one apple tree of those five has remained up to now; it has been stunted since 1946, yields small fruit and has mosaic on the leaves.

In 1930 while inspecting the fruit tree nurseries Zirnitis detected the apple rosette disease on one- and two-year old apple seedlings.

The above mentioned observations encouraged us to continue our investigations of the virus diseases of apple trees. The question of combating virus diseases is urgent not only in Latvia, but in the other fraternal republics as well.

The apple viruses has been investigated since 1953. In doing so, particular attention has been given to systematic inspection of

fruit tree nurseries and orchards, and experiments were carried out on transmission of virus diseases by grafting and by insects.

The virus diseases detected by us are as follows:

1. Mosaic disease of the apple tree (syn. Pyrus virus 2 (Bradford et Joley) Smith) occurs both on older apple trees and on one-year old seedlings. It manifests itself in the form of pale-yellow-green spots of different sizes over the whole leaf surface. Mosaic spots may be hardly noticeable or very pronounced, the manifestation depending on the age and variety of the affected apple tree, the duration of the disease and most of all – on weather conditions.

Sometimes mosaic spots are hardly to be seen. The appearance of the leaves of the young shoots seem to be normal and only by examining them closely against the light faint mosaic spots of a pale-green shade verging into the normal green colour can be detected. The spots are not pronounced and there are only a few of them on the leaves. In such cases the obvious symptom of the disease is manifested by the difference in size of leaf blades: the latter being nearly of normal size and half the normal size or even smaller on the shoots of this year and the older ones, respectively.

When the disease is of longer duration, the leaves may be entirely absent.

Sometimes the mosaic spots are much more pronounced on the ordinary green background of the leaf, and very often the spots are on either side of the mid-rib. Deformation and a faint curling of the leaves are also observed. The deformed leaves are rougher and more brittle than those of a healthy apple tree. The infection of one-summer old apple seedling usually occurs beginning late in June and continues in July and August. Seedlings diseased as a result of an early infection are greatly stunted in growth and in autumn they are not transplanted.

Seedlings infected after they have reached the required size differ little from the healthy ones and are transplanted. Mosaic spots on the transplanted infected seedlings are detected in spring.

Very often apple mosaic is accompanied by chlorosis of leaves. Symptoms of chlorosis first are manifested along the veins of the

leaf. Gradually the chlorosis increase, the leaves turn yellowish, and only the veins retain their normal green and stand out on a chlorotic background. Afterwards the veins of the leaf turn lighter, but the leaf blade becomes almost quite white, sometimes tinged with red. Rusty brown spots appear on the edges of the leaf and spread all over the surface of the leaf.

The infected transplanted seedlings are budded as well as the healthy seedlings, but they do not grow as well as the healthy ones. The following spring the buds on the diseased seedlings do not develop as well as those on the healthy ones.

The shoots are stunted in growth, mosaic spots often accompanied by chlorosis appear immediately on the leaves. The leaves reach their normal size, but gradually turn light-green. Red-brown spots appear on the leaves; the spots become confluent and the edges of the leaves curl up. As soon as the first autumn frost appears, the leaves dry up and drop off. A number of the grafted trees developed by budding on diseased seedlings die in the middle of the summer, but hardly one of them lasts through the winter. Those which last to the following summer do not develop good planting material. If left in the nursery, they are a source of infection to healthy trees. The young trees developed on healthy seedlings and infected with apple mosaic in the nursery show pronounced symptoms of the disease only after three or four years, i.e. after having been transplanted to the orchards.

Apple mosaic, as our three-year investigations and experiments show, is transmissible not only by grafting infected seedlings and by using diseased grafts - as stated in the literature - but is also transmissible from the infected apple trees to the healthy ones by the aphid, *Aphis pomi* Deg and by *Psylla mali* Schmidb. In the latter case young one- and two-year old apple trees are infected in a short time, but older trees within a few years.

It should be mentioned that we have not differentiated the apple line mosaic (apple line mosaic syn. *Pyrus virus 5* Christoff) disease from the ordinary apple mosaic disease, because (as our former investigations show) some variation in symptoms depends on the apple variety. But the investigations should be continued until a definite conclusion can be reached.

2. Bitter pit is a virus disease affecting apples. On normally developed fruit, mostly those of the winter varieties, tiny transparent blisters develops up to 0.5 mm in diameter. The skin around the blister turns reddish, but the blister itself turns into a little brownish spot which gradually increase in size and reaches from 5 to 8 mm in diameter and as much inside the fruit. The brown spots are necrotic, of a dry consistency and bitter. Sometimes the little spots appear already in spring after the petals of the blossom have dropped; in such cases the spot-bearing apples fall prematurely. In most cases the bitter pit is detected while the fruit is being kept to ripen for consumption and so causes a considerable economic loss.

The disease does not affect the growth of the tree. Sometimes hardly noticeable spots appear on the leaves of the bitter pit attacked trees, depending on the variety and other circumstances. If given only nitrous fertilizers the osmotic pressure in the leaves increases which is favourable to the development of the disease. Bitter pit is transmissible by the grafting material.

For the last two years we have observed diseased apples which show the symptoms identical to those of the rough skin (Pyrus virus 8 van Katwijk) disease. It has not yet been possible in relatively such a short time to find out whether the infection is caused by the same bitter pit virus or the virus is another one.

3. The apple rosette disease (Pyrus virus 6 van Katwijk) occurs in our republic. Some authors state that the cause of the disease is the deficiency of microelements in the soil, but others state – which is also our point of view – that the disease is caused by a virus, as it shows all the characteristics of infectious diseases. It is clearly observed in the fruit nurseries where the apple rosette disease is widely spread among seedlings. Thus, as much as 5 per cent of one-year old seedlings are affected with the apple rosette disease, but among older seedlings sometimes even 10 per cent are attacked by the disease. Up to now not sufficient attention has been paid to the infected seedlings in the fruit nurseries while budding and grafting is carried out, and they are grafted as well as the healthy seedlings. The buds inoculated on the apple rosette disease attacked seedlings do not develop. After inspection the

budding is repeated, but with the same results. The following year the same seedlings are grafted, but with the same result – the grafts do not develop. Finally, after all the grafted and budded trees are fully grown and transplanted, the diseased seedlings are also dug out and destroyed. But for the three years while in the nursery they have been spreading the virus disease.

The symptoms of the apple rosette disease are very pronounced and the affected apple trees greatly differ from the healthy ones. The leaves are considerably smaller and thicker than those of the healthy apple trees; also they are uniform in size, brittle and rough. The distance between the leaves is short, therefore the leaves look crowded; the petioles are short. The diseased leaves are paler than the healthy ones. The one-summer old seedlings are infected by the disease from the end of June till the end of August, and some have diseased leaves at the distance of 1/3 to 3/4 from the ground.

Seedlings already infected before transplantation are detected in spring, but those infected afterwards are detected only in early autumn; both have diseased leaves all over the plant and at the middle of the stem and upwards, respectively.

The apple rosette disease on one-year old apple trees is not observed as often as on seedlings.

The experiments carried out on taking buds from diseased apple trees and budding them on healthy seedlings prove the probability of transmitting the disease by budding.

The infection of fully grown fruit-bearing apple trees with the apple rosette disease is seldom observed in our republic. An eight-year old apple tree of the variety Autumn Striped affected with apple rosette disease was detected in 1953. The following two years the disease increased spreading itself on the tops of the lateral branches and on some of those inside the crown. The attacked branches yielded neither blossom buds nor shoots.

Some virus diseases of apple and Japanese pear known in Japan

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Some virus diseases of apple and Japanese pear are known in Japan, mainly from descriptions of their symptoms. The present paper describes two virus diseases:

Double working disorder of apple

The apple trees are often grafted onto the top of mature trees to change the variety when the latter are declining in growth. It is frequently noticed that some of them show poor growth suddenly a few years after the double grafting and develop symptoms like those of the root rot. Although young shoots are retarded in growth and the leaf colour turns light yellow, they later bear many flower-buds and fruits. The well-coloured fruits appear early. As the trees become older, the leaves become smaller and paler, shoots become shorter, and the trees gradually decline.

When *Malus prunifolia* is the root stock, Ralls Janet the middle part, and Golden Delicious the top scion, the intermediate stock of Ralls Janet shows no symptoms while the lower half, *M. prunifolia* root stock, shows pitting, hairy root and necrosis. The disease is easily diagnosed by the hairy root symptoms. When the root stock is of *M. sieboldii* with Jonathan in the middle and Golden Delicious the top scion the upper half shows no symptoms and the lower half shows xylem necrosis in the root stock. When the top is Starking Delicious, necrosis is shown on the transverse section of the root stock. When *M. prunifolia* is used as a root stock, the symptoms appear in virtually every case, whereas when *M. sieboldii* is used for the same purpose, the symptoms are reduced

Fig. 1. Transverse section of the root stock, which is *Malus prunifolia*, infected by double working disorder disease. Phloem necrosis appears sporadically in the vascular bundle.
(Photo: K. Ushirosawa)

by about two thirds. No symptoms are observed when the root stock is an apple seedling. When scions from the diseased trees are taken for grafting or double grafting, development of symptoms occurs to a much greater extent. In contrast, no symptoms develop when scions from symptomless trees are grafted onto *M. prunifolia*, although if scions taken from the diseased tree are grafted directly onto *M. prunifolia*, without an intermediate stock,

the stocks develop symptoms. Bark necrosis and stem pitting are distinctly visible on the root stock of *M. prunifolia*. On the root stock of *M. sieboldii*, stem pitting and xylem necrosis are usually apparant.

It is considered from the above results that this type of apple tree disorder is possibly a virus disease, and not a simple physiological disease caused by top-working.

Fig. 2. Hairy root symptoms of the root stock, which is *Malus prunifolia*, infected by double working disorder disease. Right: healthy roots. Left: infected roots.

(Photo: K. Ushirosawa)

Brown leaf spot of Japanese pear (temporary name)

This is often observed on Japanese pear, particularly on the varieties Nijusseiki, Niitaka and Yakumo, as brown and angular spots. Brown and angular leaf spots of 1 mm to 3 mm in diameter appear on the bottom leaves of infected shoots. Lower leaves of the infected shoot show more severe symptoms than upper leaves. The disease is easily transmitted by grafting, using either infected scions or infected stocks. It has been suggested that the possible presence of a vector of the disease might be determined by observing occurrence of the disease in the field. However, our investigations in this direction proved unsuccessful.

Fig. 3. Symptoms on leaves of shoot infected by brown leaf spot (temporary name) of Japanese pear (var. Nijusseiki). Upper row shows upper leaves and the top leaf on the right. Lower row shows lower leaves and the bottom leaf on the left.
(Photo: T. Noda)

DISCUSSION

Shay: What is the form of the *Malus prunifolia* and *M. Sieboldii* rootstocks used – wild seedlings or clonal rootstocks?

Hidaka: Seedlings are used.

Luckwill: It seems very likely that the decline of apples on *Malus prunifolia* stocks in Japan is due to "platycarpa latent" viruses in the scion varieties.

Have you yet indexed your varieties on *Malus platycarpa*?

Hidaka: No, this has not been done.

Dias: Have you obtained transmission of Satsuma dwarf by aphids? If Satsuma dwarf is identical with Tristeza of Citrus a plant hopper may also be able to transmit the latter disease. So far only aphids have been prooved to be vectors of Tristeza disease.

Nyland: I have obtained necrotic spots like you show in Japanese pear, when I grafted *Pyrus serotina* on Bartlett pears with stony pit. We do not think these spots are caused by the stony pit virus but by latent virus in these pears.

Researches on pear-tree mosaic

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By the term "mosaic" we generally mean a whole set of alterations which concern the leaves of pear trees (Posnette 1957, Blumer 1957), different one from the other and which do not normally occur together in nature.

During our activity, the purpose of which is to investigate the spread of the viroses in pear tree cultivations in Emilia, it has been proved that we may distinguish at least five different symptomatological kinds of mosaic; on the indicator variety "Butirra Hardy" these appear briefly as follows:

"simple chlorotic spotting": chlorotic spots generally interveinal and usually having a round form;
"veinal mosaic": chlorotic stripes concerning discontinuous portions of veins and adjacent tissues;
"ring-mosaic" (spotting): chlorotic elongated stripes and ring-shaped completely or so;
"red spotting": small red or bronze coloured spots, generally placed near the veins, which also may become necrotic;
"chlorosis, deformations and necrosis": consisting in chlorosis, blistering and deformations of the blade, that shows also the sides torn and here and there necrotic; also the leaves are smaller than normal and of an irregular shape.

All those aspects have been found on different varieties and with a different frequency of them; the first three aspects prevail by far.

The association in nature of these single alterations on the leaves of the same plant, seems, as we have said, rather rare and probably limited to the case of "chlorotic spotting" and of "ring mosaic".

In order to investigate whether the different kinds of mosaic that we have clearly identified may be associated in symptomatological wholes of two or more kinds on the same plant, we have undertaken, since last year, proper research.

We have prepared an adequate number of plants of var. "B. Hardy", that have been inoculated each with one single form of mosaic and afterwards (in spring of this year) grafted over with scions that had been taken from plants affected by other forms, in all possible combinations.

The observations that we have made up till now show that:

1. there was no protection provided by one pre-existing form of mosaic against any other form that is added later;
2. in the different associations there were cases of predominance of one kind of symptoms on another or on other ones, and no appearace whatever of new kinds of alteration. The symptoms of "necrosis and deformations" prevailed generally on the others so as to mask totally some of them, e.g., those of the "ring-mosaic";
3. the spreading of the different symptoms both in the vegetation derived from the stock and in the growth of scions proceeded at different speeds.

As we supposed from observations in the field on adult plants and on young scions, these kinds of mosaic have a different influence on the development of the plants. We have made detailed observations on the incidence of two kinds of mosaic only, namely the "veinal mosaic" (A) and "deformations and necrosis" (B) on the growth of the shoots of "B. Hardy".

We have grafted (in autumn) plants of *Pyrus betulaefolia* with virus free buds of B. Hardy and then we have inoculated them by grafting buds infected with the two different kinds of virus, in equal number. The measurements have been made at two successive times in the year following the year of grafting.

The results are shown in the following table.

Observation date	Average development in cm[1]			Development in per cent of the control	
	control	strain A	strain B	strain A	strain B
June 27th	119	96	73	81,15	61,38
Aug. 29th[2]	106	97	90	91,50	84,90
Total	225	193	163	85,70	72,00

1. simple average of 27 values

2. data obtained by the difference between the total development and that of the previous observation.

From the exposed data it results that some kinds of pear mosaic can depress the development of the plants also remarkably; but we have not yet sufficient data to affirm if it occurs as much also in regard to the production of the fruits.

DISCUSSION

Luckwill: We have observed drooping habit of certain pear varieties (e.g. Laxton's Superb) after head working. This condition is graft-transmissible but is distinct from rubbery wood disease of apple.

Posnette: Are the four types of mosaic always found independently or do they sometimes occur together on the same tree?

Canova: They occur singly and not together.

Infection of pear trees with apple rubbery wood virus

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Until now, rubbery wood virus has been considered merely as a virus of apple trees. The possibility that it might infect other fruit trees, for instance pear trees, was considered worth while investigating. This report gives an account of the experiments made on this subject from 1954 to 1960, at Changins/Nyon, Schwitzerland.

The first experiment was started in 1954. Several healthy pear seedlings were budded with rubbery wood-infected Golden Delicious. From two of these pear seedlings (I), buds were taken in summer 1956 and inserted onto three healthy pear seedlings (II), which were top-grafted two years later with healthy Lord Lambourne. In 1958, 1959 and 1960, all the branches formed from these grafts were "rubbery" on two of these trees.

On the other hand, five M IX and five M XI healthy scions were double-budded in 1957 with healthy Lord Lambourne and with buds from the infected pear seedling (I) mentioned above. In 1958 and 1959, the shoots of Lord Lambourne on four of these scions showed clear symptoms of rubbery wood. On the six other scions, the Lambourne bud did not grow. The ten control scions remained healthy (Bovey, 1959).

In 1957 also, six scions were inoculated from the three pear trees (II) mentioned above, using the same method. Five of them made a good growth, and four had very clear symptoms of rubbery wood infection in 1959. Two scions received a bud from a healthy pear seedling and remained healthy. The uninfected controls budded with Lord Lambourne alone remained also normal.

These facts suggest that rubbery wood virus can infect pear trees and maintain itself for several years in this host.

A second experiment was designed in 1959 to test whether the virus was present in orchard pear trees. Fifteen pear trees from several orchards near Nyon and Lausanne were indexed by the double-budding technique on healthy apple rootstocks using Lord Lambourne as indicator. Each indexing was replicated eight to fifteen times. In 1960, six of these trees can be considered as free of rubbery wood virus, six are infected by this virus and the results are doubtful for the last three trees. It has been noted that some of the infections from pear have given the most severe symptoms of rubbery wood ever seen by the author on Lord Lambourne.

From these experiments, it can be deduced that rubbery wood virus can and does infect pear trees and is probably widespread in pear orchards.

DISCUSSION

LUCKWILL: We have also indexed pear varieties on Lord Lambourne and have found latent rubbery wood in some of them.

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Split bark of pears

(ROUGH BARK OF PEARS)

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During the autumn 1955 a few pear trees of the variety Fondante de Charneu, growing on a small fruit farm in south-east Zeeland here in Denmark, showed pronounced splits and furrows on the bark, both on elder branches and young shoots. By careful inspection of the trees it was found that 9 of 25 trees showed a more or less pronounced split bark condition on the branches and shoots. These abnormalities had been observed by the owner during the last five years, and the affected trees seemed to be rather poor yielders. Trees of the same variety, growing next to the diseased trees, showed no symptoms at all and the whole appearence of the affected trees very much indicated a virus infection.

For closer examination of the disease a topworking experiment was initiated in the spring of 1956. Two of the most affected trees were topworked with healthy scions of the following pear varieties: Bonne Louise, Clara Frijs, Conference, Doyenne du Comice, Fondante de Charneu, Grev Moltke, Nouveau Poiteau, Tongre and Williams.

A few branches on each tree were left ungrafted as a control on the original symptoms.

The grafted scions of all 9 varieties grew well in both trees and already in the spring of 1957 – in other words after one growing season – we found distinct split bark and rough bark symptoms in 2 of the ingrafted varieties, namely Fondante de Charneu and Nouveau Poiteau. The other 7 varieties showed no symptoms on the shoots after one growing season.

The following year, in the spring of 1958, we found splits in

Splitbark of pears. Affected branches of the variety Fondante de Charneu. Characteristic symptoms. Foto: M.H.D.

the shoots of Williams. In the spring of 1959 the variety Tongre showed rough bark on the shoots – but no splits on branches or shoots occurred in this variety.

Now it is more than five years since this experiment was started – and the result so far shows that infected Nouveau Poiteau react with very severe split and rough bark symptoms in all the scions – in fact with much more severe symptoms than those on the originally diseased Fondante de Charneu.

At present the topworked Fondante de Charneu and Williams show symptoms like the originally diseased Fondante de Charneu.

The varieties Grev Moltke, Bonne Louise, Doyenne du Comice only show medium split bark and rough bark symptoms. Only rough bark, not splits have been noticed in the variety Tongre. The varieties Conference and Clara Frijs have shown no symptoms at all.

Another transmission experiment was started in Aug. 1957 when affected material of Fondante de Charneu and healthy buds of 8 different pear varieties were budded into pear seedlings. Also this experiment showed that Charneu and Williams are very sensitive, and furthermore that the varieties Conference and Clara Frijs apparently do not react. None of the control plants have developed any bark symptoms at all. Unfortunately we have not used the variety Nouveau Poiteau in this experiment.

The question is now whether or not the varieties Conference and Clara Frijs are infected.

By topworking the inoculated and all the control trees with healthy scions of the variety Nouveau Poiteau we hope it will be possible to obtain decisive evidence about the possible carriers.

In addition to the experiments with the different pear varieties we have tried to transmit the disease to species of Cotoneaster, Prunus, Sorbus and Amelanchier but none of these plants have shown any symptom reaction as yet. Also back transmission to pears from the different inoculated plant species has been started but these experiments are still not finished.

Split bark of pears is not a well known disease in Denmark, but by closer inspection it appears that several pear trees in the pear plantations show split bark symptoms – especially within the varieties Fondante de Charneu and Williams.

It is however not known how much the disease reduces the yield, but evidently the growth of infected trees is affected.

DISCUSSION

CROPLEY: We have a similar disease of Williams' in England. The virus is carried without symptoms in B. Hardy, and affects Williams' when infected B. Hardy is used as an intermediate. We have also transmitted a similar disease from Comice to Comice.

A soil-borne virus disease of cherries in the Netherlands

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Introduction

In 1951 Mulder (4) described a virus disease occurring in cherries in the Netherlands and known there as "Eckelrader ziekte" of cherry. Later the identity of the disease with the "Pfeffingerkrankheit", occuring in Switzerland, was generally assumed. In 1959 Pfaeltzer (5) proposed the name of "rozetziekte" for the Netherlands as it was thought to be undesirable to name a disease after the locality where it was found for the first time.

Infected cherry trees display both primary and secondary symptoms. The primary symptoms are the so-called oil flecks and malformation of the leaves. The secondary symptoms consist of small, narrow, sharply dentated leaves and of enations at the lower side of some of the leaves. Eventually rosettes of leaves are visible at the end of those twigs that fail to elongate normally.

The "rozetziekte" causes considerable damage in the orchards of the province of Limburg. As the disease was and still is spreading in that area the danger existed that the virus might spread to northern parts of the country. For these reason it was considered to be important to know how the virus spreads in the field. Evenhuis (2, 3) carried out a number of experiments to establish whether insects, particularly leafhoppers, were able to transmit the causal virus. These experiments, however, all gave negative results. Blumer (1) and others suggested that the virus might be transmitted by root contact. In the following section a number of experiments are described in which we investigated whether soil-transmission is indeed possible.

Experiments on soil-transmission of the virus

I. In Limburg

Three experiments were carried out with seedlings of *Prunus avium* in a diseased orchard in Limburg. In the following paragraphs these experiments will be described. The results are summarized in table 1.

Experiment A aimed to prove the infection of healthy seedlings when they are planted in an infected orchard. No special measures were taken to prevent the roots from touching the roots of diseased trees, by which possibly root grafting might take place.

In experiment B seedlings were planted in drums filled with healthy and infected soil respectively. Moreover a number of seedlings were planted in the orchard itself. In the drums contact with roots of trees growing outside the drums was of course not possible.

In experiment C ten healthy seedlings, planted in pots were brought from Wageningen to Limburg and placed at random in the orchard to expose them to air-borne vectors. After about one month the plants were changed for fresh ones, whereas the old ones were taken back to Wageningen. Here they were kept in pots, to see whether they had become infected. This was done five times during the summers of 1957 and of 1958. Thus 120 seedlings were used in this experiment.

Table 1 gives the rates of infection in each group of seedlings.

Table 1. Transmission of the "rozetziekte" of cherry under natural conditions

	Experiment	Number of test plants	Number of infected plants
A	in orchard (root contact possible) . . .	9	7
B	in drums with infected soil	20	5
	in drums with healthy soil	20	0
	in orchard	20	8
C	in pots (contact with air-borne vectors possible)	120	0

From the symptoms they showed in 1960 it can be concluded that infection of seedlings occurred only when they had grown in infected soil.

II. At Wageningen

In an experiment carried out at Wageningen we used drums filled with infected or uninfected soil, which had been treated in different ways (fig. 1). The treatments were as follows:

Fig. 1. Experiment on soil transmission of the virus of "rozetziekte" of cherry (= Eckelrader disease). Seedlings of *Prunus avium* are planted in infected or uninfected soil which was treated in different ways.

A. Uninfected soil.
B. Uninfected soil to which roots of diseased trees had been added.
C. Infected soil.
D. Infected soil, sterilized by steaming.
E. Infected soil treated with DD and subsequently aerated by means of spreading and turning at set times during several weeks to evaporate DD.
F. Infected soil treated as E. but without DD.

In each drum three *Prunus avium* seedlings were planted. Each treatment comprised eight drums.

Table 2. Soil transmission of the "rozetziekte" of cherry into seedlings growing in soils treated in different ways

	Conditions of the soil	Treatment	Number of infected plants per drum								
A	uninfected	—	0	0	0	0	0	0	0	0	0
B	uninfected	diseased roots added	0	0	0	0	0	0	0	0	0
C	infected	—	2	2	3	1	3	1	0	3	15
D	infected	sterilized by steaming	0	0	0	0	0	0	2	0	2
E	infected	DD added, then aeration	0	0	0	0	0	0	0	0	0
F	infected	aeration without DD	2	0	0	0	0	0	0	0	2

The results of this experiment, given in table 2, show clearly that untreated, infective soil brought about infection in many of the cherry plants growing in it. The only case in which infection occurred in the sterilized soil must be attributed to incomplete sterilization. No infection took place in the DD-treated soil and in only one drum did infection occur in treatment E, comprising turning and spreading of the soil. Soil taken from an infected orchard keeps its infectivity for at least some months, but it is clear that disturbing the soil (treatment F.) decreased the infectivity considerably.

A plausible conclusion from the results of this experiment is that the virus is transmitted by an organism living in the soil, although adsorption of the virus to the soil particles cannot be excluded.

The nature of the vector is not yet known. It may well be an eelworm as is known to be the case with other soil-borne viruses. Experiments are in progress to investigate this possibility, but these results can only be expected, at the earliest, next year.

In all transmission experiments described above many of the trees not only showed primary symptoms, but also secondary symptoms (fig. 2).

Fig. 2. Symptoms of "rozetziekte" of cherry (= Eckelrader disease) on a seedling of *Prunus avium* growing in untreated infective soil (treatment C). The top leaves show primary symptoms: malformation and oil flecks. The lower leaves show secondary symptoms: They are small, narrow and sharply dentated.

In the orchard we have never found any cherry tree which has developed year after year primary or secondary symptoms only. It seems therefore reasonable to assume that the disease is caused by a single virus and not by a complex of viruses.

In deciding whether or not infection has taken place, we have to rely on the production of symptoms by the plants because there is no method to demonstrate infection until the symptoms become visible. To be sure that a plant did not become infected we have to wait for at least two years.

Hosts of the virus

As was described earlier, it takes a long time before one can establish, by planting healthy seedlings, whether the soil is infected or not. We have therefore tried to find an indicator which might enable us to demonstrate an infection of the soil within a short time. For that purpose several rosaceous plants were planted in an orchard, which was known to be severely infected.

Table 3. Natural infection of different plant species by the "rozetziekte" of cherries

Plant species	Number of test plants	Infected test plants
Morello	10	3
Peach	17	0
Myrobalan	9	0
Prunus mahaleb	4	1
F 12/1 root stock	6	5
Raspberry	5	1
Blackberry	3	2

In table 3 the results of these experiments are given. Morello, *Prunus mahaleb*, F 12/1 root stock, raspberry and blackberry became infected. The symptoms of the infected plants, however, take as long a time to develop as when cherry seedlings are used. They therefore are no better indicators than cherry itself, yet they are of importance because they have to be regarded as possible sources of infection in the soil.

In greenhouse experiments certain herbaceous plants such as tobacco, cucumber, spinach, turnip, beet, plantain and other species were tested for their qualities as indicators. So far no useful results have been obtained.

Identity of the virus

It is very difficult to say whether there is a relationship between the cherry virus described here and the cherry viruses and soil-borne viruses described elsewhere.

Identification of such viruses by comparison of symptoms in cherry trees is impossible in most cases as the exchange of infected plant material is hampered by quarantine measures. Moreover the development of symptoms in trees often takes a long time. For this reason, transmission of the virus involved to herbaceous hosts might be helpful. The virus could be transmitted from cherry to tobacco and cucumber plants and from those to many other herbaceous hosts in all of which the virus became systemic.

In order to prove that the virus involved was indeed the virus which causes the "rozetziekte", inoculations back to cherries have been carried out (6). Cherry seedlings were inoculated by rubbing the young leaves with infectious tobacco sap and carborundum or by injecting the inoculum into the stems. The speed of development of symptoms varied considerably depending on the age of the plants, the mode of inoculation and seasonal influences. The shortest incubation time found was about four weeks; the longest 15 months. The test plants showed typical chlorotic flecks and ring-spots in some of the leaves but until now none of the secondary symptoms could be detected.

The question arises whether in these and in other experiments the cherry ring-spot virus might be involved. This is, however, very unlikely as there has never been any virus transmission to tobacco or cucumber from cherries showing ring-spot symptoms. The chlorotic flecks and rings which developed in the cherry seedlings inoculated with infectious tobacco sap can therefore not be attributed to the cherry ring-spot virus.

The symptoms caused by the virus from "rozet"-diseased cherries in herbaceous hosts vary a great deal. Other viruses such as raspberry ring-spot and raspberry yellow dwarf, which might be related to this virus, cause similar symptoms in one host and differing symptoms in another.

For this reason identifying cherry viruses and determining their relationship with other viruses by means of comparing

symptoms in herbaceous hosts, encounters serious difficulties. Serology will give us a better basis for answering the questions concerning the identity of the virus of the "rozetziekte" of cherries and its relationship to other viruses.

SUMMARY

In 1951 Mulder described the "Eckelrader ziekte", a virus disease of cherries in the Netherlands. In 1959 Praeltzer proposed to name the disease "rozetziekte" of cherries. In experiments concerning the transmission of the causal virus no air-borne vector could be detected by Evenhuis.

A number of experiments described here point to the fact that the virus is soil-borne (tables 1 and 2). Experiments are in progress to investigate whether eelworms can transmit the virus.

The virus of the "rozetziekte" infected morello, *Prunus mahaleb*, raspberry, blackberry and rootstock F 12/1 when grown in infected soil under natural conditions (table 3). Alle the plants mentioned developed symptoms only a long time after planting into infective soil and therefore they cannot be considered as good indicator hosts. However, they can be regarded as possible sources of the virus in the soil.

A number of herbaceous plant species could be infected mechanically with the virus, viz. tobacco and cucumber. Inoculation of the virus from tobacco back to cherries caused chlorotic spots and ring-spots in some of the cherry leaves, but secondary symptoms have not yet been observed.

Herbaceous hosts can provide suitable material for the preparation of antisera. The use of antisera may be of great help in establishing relationship amongst a number of the soil-borne viruses known at present.

DISCUSSION

Baumann: To avoid confusion in nomenclature clearly differentiation between the virus disease and the causing virus must be made.

Therefore we should not name the well known Eckelrader ziekte as rosette ziekte, because in this disease according to Cadmanns statement not only the cherry rosette virus is involved.

Furthermore the name is not specific for the main symptoms of Pfeffinger and Eckelrader disease and already used for another virus disease of sweet cherry (Blumer 1953).

KEGLER: Did you investigate samples of soil from highly infected orchards, and did you find any free living nematodes such as Xiphinema sp.?

PFAELTZER: We inspected samples from highly infected orchards and found free living nematodes, e.g. a species of Longidorus, but no Xiphinema was found.

SCHMID: Neither have we found any Xiphinema in Pfeffinger diseased soils.

MILLIKAN: Did you find enations on leaves of Prunus mechanically inoculated with juice?

When did you expect secondary symptoms to occur?

PFAELTZER: No secondary symptoms have been detected up to now.

GUALACCINI: Did you for the inoculations of the disease into herbaceous host plants use leaf juice only or juice together with any buffer solution?

PFAELTZER: Only carborundum was added to the plant sap and no buffer was used.

NYLAND: Did you make isolation attempts from roots of herbaceous plants which you grew in infected soil?

PFAELTZER: Yes, but I obtained no transmission.

KUNZE: There are some latent viruses in cherries, which are sap-transmissible to cucumber and tobacco, but the longevity of these viruses is shorter than that of the "Pfeffinger virus". It is therefore difficult to transmit such viruses from cherries to herbaceous plants unless buds are used as inoculum.

RASKI: A recommendation is made to use the term *migratory plant parasitic nematodes* when referring to the ectoparasitic forms found transmitting virus diseases. Several contributors have used the term "free living" plant parasitic nematodes, which will be confused with the large group of nematode species found in almost all cultivated fields. These have traditionally been called "free living" since they fed on bacteria, fungi and other microorganisms but not higher plants.

MILLIKAN: Have the seedlings which were infected with tobacco sap shown secondary symptoms?

PFAELTZER: Up till now no secondary symptoms have been found on inoculated cherry seedlings. As the secondary symptoms always appear some time after the primary symptoms it is possible that they will develop next year or even within two years. If they do not appear at all that will confirm the hypothesis of the complex character of the disease.

POSNETTE: How many trees were inspected on their symptom expression? May be some trees in the field might show only one type of symptoms.

PFAELTZER: An elaborate investigation on the spread of the disease in orchards of southern Limburg has been carried out by the Plant Protection Service during several years. During this investigation no trees

were found which year after year developed either primary or secondary symptoms only. Of course a tree may show only primary symptoms during the first phase of the disease after infection and during the last phase it may show only secondary symptoms. From this we can draw the conclusion that the factor (or factors) which causes (cause) the two types of symptoms is (are) always present in infected trees.

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Soil-borne viruses in stone fruits and other fruit crops

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Perusal of the literature on virus diseases of fruit trees tends to lead to bewilderment and one grasps, in the main, only two points. First, the majority have no known means of spread, other than artifical ones such as grafting; secondly, some of these diseases are characterised by leaf symptoms of the ringspot type. These are slim facts on which to base any hypothesis. But they make soil-transmission worth considering as one of the modes in which viruses spread in fruit trees because soil-borne viruses are now known to cause diseases of the ringspot type in raspberry, strawberry, grape vine and other crops, whose mode of spread was previously unknown. Some support for the idea that such viruses occur in fruit trees comes from work on peach yellow bud mosaic disease. This is soil-borne (36) and caused by a virus of the ringspot type, tomato ringspot virus (7) which has an ectoparasitic nematode, *Xiphinema americanum*, as a vector (3). Other soil-borne viruses which share many properties with tomato ringspot virus occur widely in Britain and continental Europe and there is already much evidence to suggest that at least one of these causes a severe disease in sweet cherry (*Prunus avium* L.). Several properties of these viruses make them difficult to identify and it therefore seems worth summarizing current information about them and, in particular, establishing the identities and relationships of those which have been isolated from sweet cherry in Europe.

Four soil-borne viruses of the ringspot type have been discovered. These are arabis mosaic, raspberry ringspot, tomato black ring and tomato ringspot viruses. They are called ringspot viruses because all infect tobacco (*Nicotiana tabacum* L.) plants systemically and produce diseases resembling tobacco ringspot from which

the plant subsequently 'recover' producing apparently healthy but virus-containing leaves. These four viruses all have 'spherical' particles of the order of 300 Å diameter (16, 18) and share many other biological and physical properties but they appear to share no antigens either with each other or with tobacco ringspot virus itself (Table 1). Each infects a wide range of herba-

Table 1. Physical properties of soil-borne ringspot viruses

Virus	Source of infective sap	Thermal end-point °C	Dilution end-point	Longevity in vitro (days)	Authority
Arabis mosaic	*Chenopodium*				
Grape vine strains	*amaranticolor*	58—65	10^{-3}–10^{-4}	14—21	(5)
Strawberry & other	*Petunia*				
strains	*hybrida*	55—61	10^{-4}	14	(12)
Raspberry ringspot	*Nicotiana*				
	rustica	65—70	10^{-4}	14	(13)
Tomato black ring	*Nicotiana*				
	tabacum	63—66	10^{-3}	14—21	(10)
Tomato ringspot	*Petunia hybrida*	58—60	10^{-4}	5—7	(7)

ceous and woody plants, producing ringspot symptoms in many, and this has led to the same virus being given different names when isolated from different hosts on different occasions (Table 2). Thus, arabis mosaic virus, first described by SMITH & MARKHAM

Table 2. Some diseases in crop plants associated with soil-borne ringspot viruses

Host	Disease	Virus
Peach	Yellow bud mosaic	Tomato ringspot
Raspberry	Leaf curl	Raspberry ringspot
	Yellow dwart	Arabis mosaic
Rhubarb	Mosaic	Arabis mosaic
Strawberry	Mosaic Yellow crinkle	Arabis mosaic
Sweet cherry	Pfeffinger	Raspberry ringspot
	Eckelrader	Raspberry ringspot Cherry leaf roll[1]
Vine	Fanleaf Urticado	Arabis mosaic

1. Evidence that this virus is soil-borne is equivocal.

(34) from *Arabis,* was later isolated from raspberry and named raspberry yellow dwarf virus by Harrison (12). The same virus was called rhubarb mosaic virus by Klinkowski (20) and strains which infect grape vine are known as fanleaf in California and by a variety of other names in Europe (4, 5, 31). Tomato black ring virus, described from tomato in England (33), was named beet ringspot in Scotland (10) and potato bouquet virus in Germany (21). More recently, the Californian peach yellow bud mosaic virus (37) has been identified with tomato ringspot virus (7). All these viruses produce such similar symptoms in herbaceous test plants that serological and/or plant-protection tests are the only infallible means of distinguishing the viruses from one another.

Ectoparasitic nematodes of the Dorylamoid group are vectors of arabis mosaic, tomato black ring, tomato ringspot and probably also of raspberry ringspot viruses (17, 15, 25, 3). Typically, both the viruses and their nematode vectors seem patchily distributed in soils and to persist indefinitely in fields where they occur. There is little information on why this is or on how the viruses and nematodes spread from one locality to another. Like tomato black ring virus (6) probably all the viruses can be introduced to 'receptive' soils, i.e. soils containing the appropriate nematode vector, and can be disseminated through the use of infected planting material. On the other hand, all the viruses are seed-borne in several of their hosts (24, 19) and dissemination in infected weed seeds may well be a more natural and efficient means of virus spread.

As pathogens of fruit trees, tomato ringspot virus is probably the best known, for it causes yellow bud mosaic of peach in California and has been transmitted to several other kinds of stone fruits (35). The nematode vector, *Xiphinema americanum*, occurs in Europe (5) but the virus has not certainly been found there.

Harrison (11, 14) found that peach and mazzard cherry (F 12/1) trees became infected by tomato black ring virus when grown in infective soil in the glasshouse. Peach trees developed symptoms resembling those of yellow bud mosaic but the cherry trees showed no symptoms. There is no evidence yet of tomato black ring virus naturally infecting fruit trees.

Arabis mosaic virus was isolated by Posnette and Cropley (29) from trees of three varieties of sweet cherry with rasp-leaf symptoms in England (4). This virus occurs widely in southern England and on the Continent, but its role as a cause of rasp-leaf disease in cherry is still uncertain.

Raspberry ringspot virus seems of major importance as a pathogen of sweet cherry in Europe because it is associated with diseases of the rasp-leaf type in Germany, the Netherlands, Switzerland and Britain. Kunze (22) found that cherry trees affected by Pfeffinger disease in the Rhine Valley in Germany always contained a sap-transmissible virus which he was unable to detect in comparable healthy trees. The properties of this virus resembled those of raspberry ringspot virus and serological tests showed that the German virus was closely related to English strains but more distantly to Scottish strains of raspberry ringspot virus (4). Strains of raspberry ringspot virus were later isolated from diseased cherry kindly sent me from the Basle district by Mr. G. Schmid, Wädenswil, and from the Netherlands by Miss H. J. Pfaeltzer. In England, a virus isolated from Bing cherry grafted with scions from a Napoleon Bigarreau with rasp-leaf symptoms collected in Kent was also identified with raspberry ringspot virus (29, 4). Recently, Schuch & Mischke (32) at Heidelberg have described a virus isolated from sweet cherry with Pfeffinger disease which, from its properties, may well be raspberry ringspot virus but this identity is unestablished.

Kunze's observations are perhaps the best evidence so far that raspberry ringspot is the cause of Pfeffinger disease as it is known in Switzerland (2) and Germany, because the virus seems always present in diseased trees and absent from healthy ones, but the critical experiments which would confirm this have yet to be made.

Speculation on the etiology of the Eckelrader disease of sweet cherry in the Netherlands, described by Mulder (26) and now re-named Rozetziekte (rosette disease) by Pfaeltzer (27), is on much less secure ground. Although the Pfeffinger and Eckelrader disease share many features, to equate them seems questionable, and to introduce another name already used to describe a disease

of sour cherry in North America (*Prunus cerasus* L.) (1, 23) only adds confusion.

From the results of tests which Miss PFAELTZER has kindly allowed me to take with her material I deduce that two viruses are concerned in Eckelrader disease. One of them is raspberry ringspot virus. Critical evidence for this came from two cherry seedlings, one of which became infected when planted in infective soil from Limburg where Eckelrader disease occurs; the other was infected by injecting sap from tobacco plants that contained a virus isolated from a diseased cherry tree. Raspberry ringspot virus was isolated from each seedling and identified serologically.

Earlier, Miss PFAELTZER had sent me several cultures of a virus which she had isolated from cherry naturally infected with Eckelrader disease. Unlike raspberry ringspot virus, it infected *Chenopodium amaranticolor* Coste & Reyn. systemically but failed to infect *Petunia hybrida* Vilm. and had a thermal end-point in infective tobacco sap of 55-60°C compared with 65-70°C for raspberry ringspot virus (13). Serological tests showed is was unrelated to arabis mosaic, raspberry ringspot, or tomato black ring viruses but was indistinguishable from a virus isolated in England from sweet cherry with leaf roll disease (30) and sent to me by Mr. CROPLEY (8). An antiserum prepared to the Dutch virus had the same titre (1/128) to both the Dutch and English isolates and after the antiserum had been absorbed with 7 vol. preparation of the English isolate no antibodies to the Dutch isolate were detected. Unlike the Dutch isolate, the English one infected *P. hybrida* systemically but produced slight or no symptoms.

The simplest interpretation of these facts is that, in the Netherlands, cherry trees with symptoms of the rasp-leaf type are infected sometimes with raspberry ringspot alone and at others with this and a second virus, for which the best provisional name seems cherry leaf roll virus. This would account for the likeness between the Eckelrader and Pfeffinger diseases as well as for the variability in symptoms in cherry observed in the field and perhaps also for some of that observed in herbaceous test plants in the glasshouse.

Raspberry ringspot virus is soil-borne (13) but there is no unequivocal evidence that this is true of cherry leaf roll virus. By

implication it may be, but, as no critical evidence is available, it is impossible to guess which of the viruses have been transmitted either through the soil or mechanically to cherry in the Netherlands (27, 28, 9).

Thus far, the evidence encourages the belief that soil-borne viruses play a role in fruit tree pathology and it seems not unlikely that the viruses mentioned here may occur and cause disease in fruit trees other than cherry and peach. Little further work seems needed to resolve the etiology of the Pfeffinger and Eckelrader diseases but the need to use infallible means of identifying the viruses concerned cannot be over-stressed.

SUMMARY

Diseases of the ringspot type, whose mode of spread is unknown, are common in fruit trees and it is suggested that some of these may be caused by soil-borne viruses. Evidence that four such viruses, arabis mosaic, raspberry ringspot, tomato black ring and tomato ringspot viruses, infect fruit trees is reviewed and some speculations made on the role of raspberry ringspot virus in the Pfeffinger and Eckelrader diseases of sweet cherry (*Prunus avium*).

DISCUSSION

Millikan: Does the ringspot group include the stone fruit ringspot viruses?

Cadman: No, I think that viruses of the tobacco ringspot group should be clearly distinguished from those of the stone fruit necrotic ringspot viruses.

Baumann: Can Dr. Cadman make some remarks on the relation of tobacco ringspot virus and raspberry ringspot virus? Are they identical or only closely related?

Cadman: There is at present no evidence that the North American tobacco ringspot virus has any relationship with any of the soil-borne ringspot viruses so far described from Europe.

Hewitt: It is my opinion that the virus diseases of grape vines in California are similar to those described in European grape vines and it is therefore reasonable to assume that the causing viruses are also similar. They may be related to viruses which cause diseases of other plants such as those caused by Arabis mosaic virus as shown by Cadman.

Schmid: Would you be able to prove serologically the difference between Pfeffinger disease with and without Necrotic ringspot virus?

Cadman: No, only the common component, raspberry ringspot virus, could be identified serologically.

MILBRATH: What is the significance of serology when there is such a variation in the degree of activity between the same virus in different countries?

CADMAN: Antigen excess can prevent serological precipitation – especially with the small spherical viruses. This is a factor to be recognised when negative results are obtained. Failure is not always attributable to low virus content.

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Some viruses transmitted by sap inoculation from cherry trees with European rasp leaf and leaf roll diseases

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Abstract

Buds or young leaves from sweet cherry trees were macerated in four times their own weight of either phosphate buffer or 0.01 M sodium diethyl-dithiocarbamate, and rubbed onto leaves of young herbaceous plants.

Raspberry ringspot virus was transmitted from rasp leaf diseased trees in several orchards, but not from healthy trees. From trees with rasp leaf symptoms in three other orchards arabis mosaic virus (raspberry yellow dwarf virus) was transmitted. The identity of these viruses were confirmed by plant protection and serological tests.

A virus was transmitted from cherry trees with leaf roll disease to tobacco, *Chenopodium amaranticolor* and cucumber. The virus was transmitted by sap inoculation from cherry to *Prunus pennsylvanica,* and from tobacco to *P. pennsylvanica* and *P. avium* seedlings. Antiserum to this virus gave specific precipitation lines in gel-diffusion tests when cherry sap from infected trees was used as the antigen. This virus is related to a virus isolated by Miss Pfafltzer from cherry trees with rosette (Eckelrader) disease in Holland (Cadman, personal communication). In gel-diffusion tests Cadman's rosette antiserum precipitated the leaf roll virus in *Chenopodium* and cherry sap, and the East Malling leaf roll antiserum precipitated rosette virus in *Chenopodium* sap.

No final interpretation of these results can be made until all

three isolated viruses have been transmitted back to cherry. In England raspberry ringspot virus apparently causes symptoms similar to those of Pfeffinger disease while arabis mosaic virus produces milder symptoms of the same type.

Some symptoms of the English leaf roll disease are similar to the Dutch rosette (Eckelrader) disease (assymmetrical growth of the leaves, deeply indentated margins and rosetting), but leaf roll trees do not have 'oil flecks' or enations on the leaves. The viruses isolated from leaf roll and Eckelrader diseased trees, although serologically related, may be sufficiently different in their pathogenicity to cause either leaf roll or rosette diseases. Alternatively, another virus, such as raspberry ringspot, may be necessary for the production of the enations in the Eckelrader syndrome.

Rasp leaf

Rasp leaf disease of sweet cherry was first described by Bodigne and Newton in America. Diseases characterized by similar leaf enations have been reported from Switzerland (Pfeffinger disease), Holland (Eckelrader disease), Germany and England. In England the name rasp leaf has been used to avoid a multiplicity of names for diseases with similar symptoms.

Young leaves from orchard trees with rasp leaf symptoms, and from Bing trees infected by bud-inoculation, were macerated in an alkaline phosphate buffer or in 0.01M sodium diethyl dithiocarbamate, and the sap rubbed onto the leaves of young *Chenopodium amaranticolor, Petunia hybrida* and White Burley tobacco plants. Raspberry ringspot, a soil-borne virus causing local lesions on *Chenopodium* and systemic symptoms on *Petunia,* was transmitted from infected trees in seven orchards, but not from healthy trees. Tobacco plants rarely became infected when inoculated with infective cherry sap, but were readily infected with *Petunia* sap. The identities of the isolates were confirmed by agar gel-difussion serological tests, using undiluted sap from infected *Petunia* plants. These tests were reliable during the winter months, but during the summer the virus titre was sometimes too low to give reaction.

Arabis mosaic, a soil-borne virus transmitted by the nematode *Xiphinema* was isolated from trees with small enations in three other orchards. Arabis mosaic was also transmitted from an *Astilbe* plant that had deeply serrated leaves with enations on the undersurfaces.

No very consistent differences in symptoms of raspberry ringspot and arabis mosaic infected trees are yet known. 'Oilfleck' symptoms have been seen on both raspberry ringspot and arabis mosaic trees, but there is an indication that trees infected with raspberry ringspot have large enations, with narrow tough leaves, while arabis mosaic trees have small enations and the leaves are rarely narrow.

Leaf Roll

Leaf roll disease is a common cause of deterioration of sweet cherry trees and the virus has been isolated from twelve orchards in England. In some orchards only one or two trees are infected while in others the disease is prevalent. (Posnette and Cropley 1955).

A virus was consistently transmitted from infected trees to tobacco and *Chenopodium*. Lesions often developed on inoculated tobacco leaves within 48 hours; systemic symptoms on *Chenopodium* were similar to those caused by Arabis mosaic virus. The virus was transmitted to other herbaceous hosts, including cucumber, French bean, sugar beet, all of which developed symptoms, and *Petunia* and *Vinca rosea* seedlings which developed no symptoms but from which the virus was recovered.

The virus was precipitated from infective tobacco sap by ammonium sulphate with little loss of infectivity, but unlike arabis mosaic and raspberry ringspot viruses, precipitates formed with ethanol or acetone were not infective.

Gel-diffusion serological tests were reliable throughout the year when undiluted sap from systemically infected *Chenopodium* leaves were used as antigens. During the spring, cherry sap from buds or very young leaves was also antigenic. Antiserum to this leaf roll virus reacted with a virus isolated by Miss Pfaeltzer from Eckelrader (rosette) trees (Pfaeltzer 1959), and the English

leaf roll virus reacted with an antiserum, provided by Dr. CADMAN, to the Dutch virus.

The virus was transmitted to *Prunus pennsylvanica* and *P. avium* seedlings when infected tobacco leaves were macerated in four times their own weight of 0.01 sodium diethyl-dithiocarbamate and rubbed onto carborundum-dusted leaves. The virus was also transmitted in this way to *Prunus pennsylvanica* with sap from infected F12/1 cherry leaves. Symptoms developed on inoculated *Prunus* seedlings after 3 weeks. On *Prunus pennsylvanica* chlorotic spots, blotches, rings and lines developed on the leaves, sometimes with leaf and shoot necrosis. Gum-filled swellings developed on the stems of two plants. In *P. avium* seedlings, the virus caused yellow areas on the leaves, rather similar to 'Oil-flecks', with occasional red rings and lines. One plant that was very small when inoculated has cupped leaves with purple margins, and closely resembles the trees with leaf roll disease in orchards.

Discussion

The nomenclature of rasp leaf and leaf roll diseases is very confused.

LEWIS, in Pennsylvania and ADAMS and KESSLER in West Virginia described diseases of sour cherry which they stated resembled Pfeffinger and Eckelrader diseases of sweet cherry, and proposed the name rosette. It seems illogical to compare symptoms on sweet and sour cherry, and to suggest that they are the same disease, and we know that sour cherry trees do not develop enations when infected with Eckelrader disease. Following these publications from America, Miss PFAELTZER proposed the name rosette instead of Eckelrader for the Dutch disease, and suggested that the virus she isolated from these trees might be the rosette virus or one of a complex causing the disease. However, the virus isolated by Miss PFAELTZER is serologically related to the leaf roll virus and enations, a feature of Eckelrader disease, are not characteristic of leaf roll disease. In one experiment extremely small enations developed on a very few leaves of Bing cherry the year after inoculation, but in other varieties no enations have been

seen. These enations were not comparable to the large enations illustrated in the original description of Eckelrader by MULDER. The Dutch isolates, although serologically related to leaf roll virus, may prove to differ from leaf roll virus in pathogenicity and to cause large enations although these have not been reported in the latest publication. However, as Miss PFAELTZER suggests, the virus she has isolated may be part of a virus complex causing rosette (Eckelrader) disease. If this is so, then the Dutch rosette disease is caused by a complex of leaf roll virus and another probably raspberry ringspot virus (CADMAN).

Transmission of Pfeffinger disease to herbaceous plants

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In Switzerland the Pfeffinger disease is one of the worst virus diseases of fruit trees. As you know, this disease has the big advantage that it can be transmitted to herbaceous plants as Dr. L. Kunze described in 1958 in the Phytopathologische Zeitschrift, Band 31, pp 279-288.

I will not discuss the experiments in detail, but would like to summarize some of the important facts, which resulted from this work. Mechanical transmissions from diseased cherries to herb. plants are made by grounding up cherry leaves or opening buds in a mortar under addition of phosphate buffer solution (Sörensen, pH 6,5). With a sterile cheesecloth pad the juice is then lightly rubbed on the leaves of herb. plants which have been dusted before with carborundum powder (mesh 400). After that the leaves are washed off with water. The amount of buffer solution used is not important as long as there is enough juice to rub on the leaves.

In the last three years for this type of work about 5000 herb. plants have been used. When different workers try to compare the results with each other it should be clear that we all use the same kind and variety of herb. plants. A very important factor is the temperature at which the herb. plants are held after inoculations. I don't think that reasonable comparison of symptoms on herbaceous plants can be made without regarding this factor.

Nicotiana tabacum, N. xanthi glutinosa, N. rustica, tomato and petunia are very sensitive to temperature in the symptom expression. There is a difference of symptoms due to the tempera-

ture on the inoculated leaves and then again on the newly formed leaves. For example on *N. rustica*, when the inoculum is taken from a source at 20°C the rubbed leaves will only show small, thin necrotic rings, sometimes hard to see. On the other hand when the plants after inoculations are held at 15°C, the rubbed leaves show strong transparent spots. The differences in symptoms on the newly formed leaves are even better. The leaves of the plants held at 15°C will show strong necrosis and rings and are usually deformed. On Nicotiana plants held at 20°C we may hardly see any symptoms at all or probably only fine necrotic lines or rings. Plants held at 15°C during the night and 20°C during the day showed an intermediate type of symptoms. *Chenopodium quinoa, C. amarcnticolor, C. album* and greenhouse cucumbers are not as sensitive in symptom expression due to temperature as are species of Nicotiana. In fact cucumber will not grow normally at 15°C and should therefore be held at higher temperature.

The symptom expression is also influenced by the age of plants when they are inoculated. *N. tabacum, N. xanthi glutinosa, N. rustica* should be used, when they are in the 4 to 5 leaf stage. If they are too young, they will show fairly good symptoms but not as good as in the 4 to 5 leaf stage. Old plants will not show any symptoms at all or only very faint ones.

The period when cherry leaves from diseased trees in the field can be taken for transmission experiments to herb. plants is quite long. Diseased dormant cherry twigs can be cut by the end of November and put in water and held in the greenhouse. After every 2 or 3 days the water should be renewed and the base of the twigs freshly cut. After 2 or 3 weeks the buds start to push out. From the opening buds transmissions can easily be made. Very good transmissions can also be made from mature cherry leaves when they are taken shortly after bloom until one month later. When the whole tree is diseased, it does not matter whether the basal leaves or the tip leaves of the twigs are taken.

On large older trees, which are newly infected with Pfeffinger disease and showing symptoms on only a few branches, it may be possible to cut twigs which are not diseased. From such a tree, which showed symptoms on the lower part only but no symptoms

on the top, transmissions were made mechanically to herb. plants and by budding to cherry seedlings. Transmissions from the upper parts of the tree were negative. This indicates that in large trees the virus does not move very rapidly.

In 1959 transmissions to herb. plants from cherry leaves were made until the middle of July. During the later samplings many plants did not show any symptoms at all and others only weak ones. This may be due to the higher temperature in the greenhouse or to an inhibiting factor in the cherry leaves (CADMAN). Transmissions from cherry to herb. plants should therefore be made before the month of July.

After picking cherry leaves from the trees they can be stored at room temperature in polyethylene bags for 10 days without appreciable inactivation of the virus. It may be possible that cherry leaves can be deep frozen and later be used for transmission experiments. In one case such a test was successful. Cherry leaves from diseased *P. mahaleb* were picked on the 21st of July and put in polyethylene bags at 20°C below zero. Transmissions from these leaves were made 6 months later. It was not possible to recover the virus from leaves of sweet cherries handled in the same way. It may be that these leaves were picked too late or there may be an inhibiting factor involved that is not present in *P. mahaleb*.

There is a great difference in symptom expression on herb. plants, when the inoculum is taken from different trees. In order to make good comparisons of the symptoms on herb. plants it is important that after inoculation they are held at the proper temperature. For each inoculum source at least 5 herb. plants of the same kind and variety should be used. When the inoculum is taken from cherry leaves, the symptoms on herb. plants may vary in severity. This may be due to the inhibiting factor in the cherry leaves. It is important therefore to make further transmissions from these herb. plants to other herb. plants. After the second transmission the symptoms will be stronger and more uniform. The symptoms from these plants can be compared more accurately with each other. Transmissions from herb. plants to herb. plants can be made all year round. This is determined by environmental conditions.

When we compare symptoms on herb. plants from different inocula, it can be done on the inoculated leaves, but even better on the systematically infected newly formed leaves. In *C. amaranticolor, C. album* and *C. qurnoa* both kinds of leaves must be checked; the ones which are rubbed and the newly formed ones. In *Petunia hybrida* the variety used is important. Not all varieties will show the symptoms to the same degree.

When different sources are compared, they may be similar on one species of plant but quite different on another species. It is therefore important when we try to compare the different sources with each other that we use a standardized host range for each source.

In table 1. is shown the differences in symptoms obtained, when the inoculum was taken from eight different source trees. From three source trees inoculations by budding were made in late summer to 12 one-year-old, vegetatively propagated cherry clones. The symptoms on the trees were checked the spring after inoculation. From the inoculated clones transmissions were again made to herb. plants. The herb. plants showed the same symptoms as when the inoculum was taken from the original source trees. From each of the 8 source trees inoculations were made to shirofugen flowering cherry. Buds were taken from 4 different parts of each tree. Some trees were positive for ringspot but others negative. It is concluded, therefore, that the ringspot virus is not a required component of the virus complex of Pfeffinger disease.

When we regard the different symptoms which appeared on the various herb. plants and on the cherry seedlings we see that we are dealing with a virus complex. Further experiments have to be made in order to say whether we are dealing with different viruses or just different strains of the same virus. Source 534 is quite different from all the other sources and may be a separate virus. The symptoms which appeared on the original source tree and the symptoms on the infected one-year-old cherry clones are very similar to rasp leaf as it is decribed from the U.S., except that this culture gives a very strong vein clearing symptom in addition to rasped leaves.

On older trees it is sometimes hard to see a clear cut difference between the various strains of Pfeffinger virus. They can be

Table 1. Differences in symptoms when the inoculum is taken from different source trees

Source tree	N. rust.	N. xanthi. glut.	Petunia hybr.	Chenop. amarant.	Shiro-f. RS	Symptoms on cherry clones (inocul. 7.8.59) 23.5.60	17.6.60
122	AA	AA	AA	AA	—		
123	AA	AA	AA	AA	—	123 Typical oil spots, no sharp margines, deformed leaves	Enations
124	AA	AA	AA	AA	—		
126	AA	AA	AA	AA	+++		
184	B	A	BB	AA	—	184 Similar to oil spot somewhat sharper marg.	Light deformations of leaves, enations
186	CCC	BBB	CC	AA	+++	186 Light mottle, R.S.	Enations
310	CCC	BBB	DDD	AAA	+—		
534	D	C	EE	BB	++	534 Only one out of 4 trees. Strong enations, narrow leaves, no oil spots (Symptoms only on one twig, just above the oculation)	Enations, vein clearing

Explanations to Table 1.

The source trees with two exceptions are sweet cherries (*P.avium*) from the Basel Land region. Nr 310 is a *P.mahaleb* inoculated with sweet cherries from the same region. Nr 534 is a *P.avium* from a different place.

The letters below the different host plants represent the different type of symptoms in systemically infected leaves.

One letter means good visible symptoms. Two letters mean strong symptoms. Tree letters mean very strong symptoms. Shiro-fugen: The reaction of ringspot virus; — = negative, + = positive.

distinguished quite readily in young inoculated trees and by the use of herb. hosts.

DISCUSSION

CROPLEY: What was the day length and light intensity in the experiments comparing different temperatures?

SCHMID: The day length was normal and no additional light was used. The greenhouse stayed shaded all the time.

NYLAND: Schmid's culture 534 is systemic in *Chenopodium amaranticolor* and similar to our raspleaf but RRSV is like our rasp leaf too, but produces only local lesions on *C. amaranticolor.*

But 534 is known to have vein clearing symptoms in addition to rasping.

SCHMID: Transmissions from sweet cherry to cherry seedlings were made from a source tree, which showed only enations but no other symptoms of Pfeffinger disease. The inoculated cherry seedlings showed after one year a very strong pattern of yellow flecking. The following year the seedlings showed again the same symptoms plus strong enations and deformed leaves.

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Untersuchungen zur serologischen Verwandtschaft des Raspberry yellow dwarf virus

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Das Raspberry yellow dwarf virus, ein Stamm des Arabis mosaic virus Smith und Markham (1944) ist von Harrison (1958), Cadman (1958/59), Lister (1958) u.a. in England, Schottland, Wales und auf dem Europäischen Festland nachgewiesen. Das Raspberry yellow dwarf virus (RYDV) hat einen großen natürlichen Wirtspflanzenkreis. Besonders interessiert das Auftreten des Virus bei Himbeere und Erdbeere, die stark geschädigt werden, und bei der Kirsche. Dort bildet das RYDV eine der Komponenten, die Viruskrankheiten vom Typ der Rauhblättrigkeit (rasp leaf) hervorrufen.

Vergleicht man den experimentell geprüften Wirtspflanzenkreis des RYDV mit dem eines in Deutschland von Klinkowski und Opel (1957) beschriebenen Mosaikvirus des Rhabarbers (RMV), so ergibt sich eine auffallende Übereinstimmung. Die wichtigsten Testpflanzen sind in beiden Fällen: *Petunia hybrida Vilm., Chenopodium amaranticolor Coste und Reyn, Cucumis*

Tabelle 1. Physikalische Daten des Raspberry yellow dwarf virus (RYDV) und des Rhabarbermosaikvirus (RMV)

	RYDV nach Harrison		RMV nach Klinkowski
	mildes Isolat von *Rubus idaeus*	virulentes Isolat von *Beta vulgaris*	
Thermaler Inaktivierungspunkt ...	55—58 °C	58—61 °C	58—60 °C
Verdünnungsendpunkt.....	10^{-4}—10^{-5}	10^{-3}—10^{-4}	2×10^{-3}
Haltbarkeit in vitro.....	6—8 Tage	11—15 Tage	4—9 Tage

sativus L., Phaseolus vulgaris L. und *Beta vulgaris L.* Auch die physikalischen Daten der beiden Viren stimmen weitgehend überein, wie aus Tabelle 1 hervorgeht.

Da Harrison mit Petunie, Klinkowski (1959) mit Bohne bzw. Gurke gearbeitet hat, können geringe Untershiede im Verdünnungsendpunkt und in der Haltbarkeit in vitro durch verschiedene Viruskonzentrationen in der Wirtspflanze bedingt sein.

Für das RYDV und RMV ist ferner charakteristisch, daß sie tief gefroren mehrere Wochen haltbar sind. Ein Teil ihrer Infektiosität geht nach Fällung mit 1/3 gesättigter Ammoniumsulfatlösung verloren, während beide Viren nach Fällung mit 30-50 procentigem Aceton hoch infektiös sind.

Um eine etwaige Verwandtschaft beider Viren zu prüfen, führten wir serologische Kreuzreaktionen mit gereinigten Antigenen durch. Die Ergebnisse zeigt Tabelle 2.

Tabelle 2. Titer der Antiseren des Rhabarbermosaikvirus (RMV) und des Raspberry yellow dwarf virus (RYDV) mit 3 Isolaten des RMV und dem RYDV

Antiserum	Antigene			
	RMV-Isolate			RYDV
	1	2	3	
Rhabarbermosaikvirus (Isolat 1)	1024	1024	512	512
Raspberry yellow dwarf virus.	128	128	256	256

Wir verwendeten 3 Isolate des RMV. 1 und 3 wurden von Rhabarber, 2 von *Rumex obtusifolius L.* isoliert. 1 und 2 sind milde Isolate, 3 ist eine virulente Herkunft. Der Serumtiter, der sich mit den einzelnen Isolaten ergab, stimmte zum Teil überein, z.B. bei Isolat 1 und 2 sowie bei Isolat 3 und RYDV, oder er differierte um eine Verdünnungsstufe, wie im Fall des RMV-Isolats 1 und RYDV. Dieses Ergebnis spricht für geringe Unterschiede in den antigenen Gruppen der geprüften Viren und damit für eine nahe serologische Vedwandtschaft zwischen RYDV und RMV. Harrison hat ähnliche geringe Unterschiede bei der serologischen Untersuchung seiner RYDV-Isolate gefunden, so daß die von uns ge-

prüften Herkünfte des RMV als Isolate des RYDV anzusehen sind.

Die serologisch festgestellten Vedwandtschaftsverhältnisse bestätigten sich im Prämunitätsversuch. Die milden Isolate 1 und 2 des RMV wurden auf je 40 Petunien, Sorte »Ratsherr« übertragen, bei denen sie deutliche Adernaufhellungen auf den jüngeren Blättern hervorriefen. Das später gebildete Laub zeigte keine Symptome, aber das Virus war darin serologisch nachzuweisen. Bei der Zweitinfektion wurden alle symptomlosen Blätter von je 20 Petunien mit RYDV bzw. Isolat 3 des RMV beimpft, die als virulente Isolate nekrotische Ringflecke und Linien auf den abgeriebenen Blättern bilden. 14-21 Tage p.i. zeigte keine der Versuchspflanzen nekrotische Symptome, während die gleichalterigen, nicht vorinfizierten Kontrollpflanzen nach 8-10 Tagen die typischen nekrotischen Symptome aufwiesen.

Wir haben außer dem Isolat 2, das von *R. obtusifolius* stammt, noch einige Virusherkünfte von spontan infiziertem *Rumex* aus verschiedenen Gegenden der Umgebung Halles serologisch geprüft. Alle Isolate reagierten nach Übertragung auf Petunie mit den Antiseren des RMV und RYDV. Auch das in Jugoslawien von Miličić und Bralić (1958) beschriebene *Rumex*-Virus, das sie uns freundlicherweise überließen, ist eine Herkunft des RYDV, wie die serologische Reaktion und die Übertragung auf verschidene Testpflanzen zeigte. In den USA haben Jale und Vaughan (1954) nachgewiesen, daß bei spontan infizierten Rhabarber und *Rumex* dasselbe Virus vorlag. In vieler Hinsicht stimmt das dort beschriebene Virus mit dem RMV bzw. RYDV überein. Da auch aus Italien (Ciferri, 1951), Belgien (Roland, 1952), Westdeutschland (Ullrich, 1955) und Holland (van Hoof, 1956) Berichte über ähnliche Viruskrankheiten bei Rhabarber oder *Rumex* vorliegen, ist damit zu rechnen, daß die Verbreitung des RYDV größer ist als bisher bekannt.

SUMMARY

Raspberry yellow dwarf virus (RYDV) and Rhubarb mosaic virus (RMV) have similar properties in vivo and in vitro. Serological precipitine reactions and premunity tests showed that the viruses are closely related. Different isolates from *Rumex obtusifolius L.* with

mosaic symptoms, also reacted with the antiserum of RYDV and RMV, for instance the *Rumex*-virus described by Miličić and Bralić in Jugoslavia. Since similar virus diseases of rhubarb and Rumex are described from USA, Italy, Belgium, Western Germany and Holland, RYDV is probably more wide spread than supposed.

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Sweet Cherry Rugose Mosaic Virus in California

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Sweet cherry rugose mosaic was originally described by THOMAS and RAWLINS in 1939. They reported the disease from several counties in northern California and from Riverside County in southern California. The disease is now known to occur in all the cherry districts of California and those of the Pacific Coast states, British Columbia, and Ontario, Canada. It is also known to occur in Utah.

Several strains of the virus are known and can be classified as mild, moderate and severe. A mild strain may be present in a tree for many years without causing appreciable damage. On the other hand, a severe strain may kill young trees and cause rapid decline of older ones. The general picture as seen in the orchard is intermediate between these extremes although many trees are seen with mild symptoms. The disease is of considerable economic importance and has been the main reason for the decline and abandonment of many orchards, particularly in Sonoma County, California.

Rugose mosaic virus has been recovered from orchard trees of cherry, peach, and almond. Artificially it has been introduced into and subsequently recovered from sweet and sour cherry, peach, almond, apricot, plum, *Prunus mahaleb,* and several other wild species of Prunus. It may be carried in a symptomless condition in peach, almond, some cherry varieties and seedlings, *P. mahaleb,* and some plums. *P. salicina* var. Burbank shows fairly good symptoms with the most severe strains if the trees are on peach root and the virus is introduced into the understock. Most plums on *P. myrobalan* rootstock rarely become infected when graft-inoculated with infected buds or scions.

Two strains of the virus have been mechanically inoculated to several herbaceous hosts. One strain has been returned from herbaceous hosts to cherry with production of typical symptoms. Results with the other strain are not yet available. Both of the strains which have been juice transmitted are of the fast-moving type. One was originally obtained from almond. Neither of these strains is commonly found in cherry or almond trees in the orchards. The type strain obtained from trees in the same orchard from which THOMAS and RAWLINS originally described the disease has not been transmitted with certainty. It is obvious that differences exist in the ease of mechanical transmission among the different strains. Whether this indicates more than just strain diferences must still be determined. So far it has been consistent that the types which move rapidly in the trees are juice transmissible and the slow-moving types are not.

When young cherry trees are bud inoculated with the various strains of rugose, two types of reactions are noted. Those inoculated with the fast-moving strains show symptoms the spring following inoculations of the previous summer or any time prior to bud break. Symptoms may be seen throughout young trees within 1 or 2 years following inoculation. Those inoculated with the slow-moving strains may not show symptoms for several years. However, the eventual damage to the trees may be as great or greather with the slow-moving type, since the spurs and lateral shoots may be killed very efficiently as the virus moves progressively upward in the tree. It is characteristic of this slow-moving type that the rate of movement upward and outward in the branches is much greater than downward. The virus moves downward very slowly which may be on the order of 1 or 2 nodes per year compared to 8 or 10 nodes per year upward. The type and rate of movement is similar to that of cherry rasp leaf virus in western United States. With the slow-moving types of rugose there may or may not be conspicuous leaf symptoms. Usually some leaves can be found with the typical chlorotic blotches and distortion, but the relative abundance of these seems to be a function of the strain more than the variety or climatic conditions. Small elm-like leaves with conspicuous veins are generally present in trees infected with the slow-moving strains. These leaves

are formed on spurs or shoots from latent buds and enations are frequently found on some of these leaves. The enations are often restricted to the area on each side of the midrib near the base of the leaf lamina. In some cases more fully developed enations can also be found. In the fast-moving strains the enations of the more conspicuous type are more abundant and may be as prominent as those associated with the rasp leaf virus. In these strains also, there is much more leaf deformity and chlorotic blotching and spotting than in the slow-moving strains. The abundant leaves with the chlorotic blotch leaf symptoms, leaf deformity, and the rapid rate of movement of the virus in the trees distinguish these strains of rugose mosaic from rasp leaf. Final determination of the relationship of the various strains of rugose mosaic to each other and to viruses of the rasp leaf and Pfeffinger disease groups must await further work such as serological, cross protection, and electron microscope studies.

The general symptoms in sweet cherry for all strains can be summarized as follows: chlorotic blotching of the leaves with twisting and distortion; necrosis of the chlorotic areas in the first-formed leaves as the season progresses; enations on the underside of the leaves; and, prominent venation of certain small leaves produced on spurs or short shoots usually originating from latent buds on the larger branches. Bloom may be delayed and fruit ripening may be delayed from a few days to several weeks. This varies from year to year and among the different strains of the virus. Mild to severe fruit symptoms may result in reduced colour, flattened, dimpled, lopsided or partially twisted, deformed fruits. The green fruit epidermis may be conspicuously roughened especially during shock stage of the disease in newly inoculated trees or in recently invaded branches in trees with older infections. There is a progressive reduction in fruiting wood due to killing of lateral spurs and branches and dieback. Trees may have a weeping or drooping appearance. In advanced cases compact tufts of leaves on spurs or dwarfed shoots arise abundantly from latent buds along the main branches. Leaves on rugose diseased trees do not seem to be abnormally long or narrow unless the genetic disease, deep suture, is also present in the tree.

Available evidence indicates slow to moderate spread in the

orchard among adjacent trees. Replanted trees in sites where diseased trees have been removed have remained healthy up to 9 years in one orchard in Sonoma County. It is not known at this time if rugose mosaic virus is soil borne. Several attempts to infect seedling trees of *P. avium* by planting them in soil removed from under diseased trees have given only negative results.

Preliminary tests with the strains of rugose mosaic virus transmitted to herbaceous hosts against antisera of raspberry ringspot, Arabis mosaic, and leaf roll viruses supplied by A. F. Posnette and R. Cropley were inconclusive. Definite reactions using the gel technique were obtained between RRSV antiserum and 2 strains af rugose mosaic from cherry and almond with virus antigen obtained from *Petunia* and *Chenopodium*. Distinct minor lines of precipitation were formed in addition to common host lines. Variable results were obtained when the tests were repeated, so at this time it is not possible to claim relationship between these viruses with any degree of assurance. Aarabis mosaic virus antisera also reacted with the strains of rugose in 1 test. Development of unseasonably warm weather greatly curtailed symptom expression and, presumably, virus concentration in the herbaceous hosts. It seems likely that this influenced the serological tests.

In 2 separate experiments rugose mosaic virus was inactivated in infected sweet cherry, Lovell peach, and *P. mahaleb* seedlings after heat treatment of growing plants for 3 weeks at 37-38°C.

There are many similarities between some of our strains of rugose mosaic and the diseases described as rasp leaf in England and Pfeffingerkrankheit and Eckelrader or Rosette in Europe. By exchange of antisera and research workers, it should be possible to sort out the relationship of these diseases and their causal viruses. Milbrath in Oregon has found what appears to be Pfeffinger disease there, so this should expedite the comparisons.

DISCUSSION

Millikan: When two strains of rugose mosaic virus are present in the same tree, is it then possible to recover both from different parts? Is rugose mosaic related to almond bud failure?

Nyland: Two different strains of rugose mosaic virus of cherry have not been obtained from the same tree. When artificial inoculations are made there has been some interference between strains.

Cherry rugose mosaic virus will cause failure of buds in almond, but this is different from the almond bud failure virus.

GUALACCINI: Does the cherry rugose mosaic virus when inoculated to almond produce any other symptoms than yellow irregular spots (for instance line pattern, flecking or vein clearing)?

By inoculating Italian almond mosaic virus to cherry seedlings no rugose mosaic symptoms were produced.

NYLAND: Cherry rugose mosaic virus produces only chlorotic spots and patches yellow to white in colour with no regular pattern.

Almond calico virus in U.S.A. does not produce rugose mosaic in cherry.

BAUMANN: Do trees infected with rugose mosaic virus and recovered from shock symptoms show shock symptoms at any time again, or do they remain in the recovery stage for the rest of their life?

NYLAND: Generally such trees show no more shock symptoms, except that some side branches or shoots from latent buds may develop shock symptoms. – These buds may not have been systemically infected (Hypothesis not proved).

STOUT: What length of time for the heat treatment?

NYLAND: 21 days.

WOLFSWINKEL: On the technique of heat treatment of virus infected buds I would like to ask whether the healthy rootstock into which the infected bud is inserted prior to heat treatment has to be in any particular state of growth when treatment is undertaken, that is, whether active growing rootstocks are more sensitive to heat injury by the treatment, than rootstocks in a more mature state of growth at the end of the growing season?

NYLAND: The treatment is best carried out near the end of the growing season but before bark stops slipping.

Pollen Transmission of Necrotic Ring Spot and Prune Dwarf Viruses in Cherry

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Transmission of necrotic ring spot virus (NRSV) through the seeds of various species of Prunus has been independently established by various workers, including Cochran (3, 4), Cation (2), Boyle (1), Gilmer (6), and others. The actual mechanism of virus transfer to seeds was not established by any of these workers, but it is clear that they tacitly assumed that seed infection had resulted from virus transfer from the female parent. Cochran (3), in establishing seed transmission of ring spot virus in seeds of mazzard cherry (*Prunus avium* L.) stated: "The amount of seed . . . coming from trees infected with ring spot is not known, nor is the percentage of ring spot which would result if the seed were taken from a single infected tree." In testing seed transmission of ring spot virus in peach seeds, Cochran (4) obtained his seed samples from an infected female parent, and Cation (2), in the experiments which demonstrated seed transmission of NRSV in mahaleb (*Prunus mahaleb* L.) and Montmorency cherry (*Prunus cerasus* L.), similarly obtained seed samples from infected female parents.

In 1957, Ehlers and Moore (5) demonstrated that pollen obtained from virus infected sour cherry trees might carry several of the Prunus viruses, including virus A (a strain of NRSV), virus B (prune dwarf virus, PDV), virus E, and "possibly" virus G (NRSV).

At our laboratory in 1958, during routine indexing of young sour cherry seedlings from breeding lines, 5 of 18 seedlings derived from a known virus-free female parent were found

infected with NRSV (7). Although these 5 seedlings were 3 years of age and had been grown in the field for 2 years prior to indexing, they had been well-isolated from other Prunus species – furthermore, natural spread of NRSV to such young seedlings was very unusual. In view of the work of EHLERS and MOORE (5), it appeared possible that infection in these 5 seedlings might have been contracted from the pollen parent, which was known to have been infected with NRSV and PDV. The experiments recounted in this paper were designed to test this hypothesis – that seed infection could be derived solely from the pollen parent.

Experimentation

(a) *In the greenhouse.* Fifty English Morello sour cherry trees (*Prunus cerasus*) were used as female parents. These trees had been propagated from indexed virusfree scionwood and had been grown in 8-inch pots in the greenhouse for a period of 4 years following 2 years of growth in the nursery. Individual trees differentiated from 25-30 blossom buds during the 1958 season.

During the period February-June 1959 each tree was individually indexed 3 times. The initial indexing was by bud-inoculation from the still dormant trees to 4 seedlings of *Prunus tomentosa* Thunb. and 2 seedlings of peach (*Prunus persica* Batsch.). As each English Morello tree leafed out, it was further indexed by mechanically inoculating the cotyledons of 4 cucumber seedlings (*Cucumis sativus* var. National Pickling) with buffered juice extracts of its young foliage. In the final indexing, buds from each English Morello were inserted in Shirofugen cherry (*Prunus serrulata* Lindl.) in early June. In all 3 types of indexing tests, each of the 50 English Morello trees was found free of NRSV and PDV.

As pollen parents, 4 Montmorency trees (*Prunus cerasus*) were used; the pollen donors had been indexed in 1957 and 1958. Three of the pollen donors, CR 6-29, CR 3-25, and 15-1-2, were infected with NRSV and PDV, while the remaining pollen donor, IS-5, was virus-free. Pollen samples from each donor were indexed for virus content on cucumber, squash (*Cucurbita pepo* var. Butternut), and watermelon (*Citrullis vulgaris* var. Charleston Cray); both

NRSV and PDV were recovered from pollen from donors CR 6-29, CR 3-25, and 15-1-2, thus confirming the report of EHLERS and MOORE. Neither virus was recovered from the pollen of IS-5, the virus-free donor.

Flowers of the English Morello female parents were emasculated prior to anthesis to prevent self-pollination and the stigmas hand-pollinated with Montmorency pollen. The English Morello trees were divided into 4 groups; each group received pollen from a single donor.

Fruits were harvested 61-69 days after pollination, and the pulp and stony pericarp removed from the seeds. Seeds were after-ripened for 143 days at 40°F and then planted in peat moss. As each seedling reached the 2-leaf stage, a small amount of young leaf tissue was removed and triturated in 2-3 drops of phosphate buffer (.05 Molar, pH 7.6) and the resultant extract inoculated to the cotyledons of 4 cucumber seedlings. Each seedling was indexed at least once, and when possible, 3 times with this technique. Identifications of NRSV and PDV were made by sub-inoculations from infected cucumber seedlings to seedlings of squash, *Tithonia speciosa,* and watermelon. Data on virus recovery are presented in Table 1. These same data are re-expressed as percentages in Table 2.

Table 1. Virus transmission through seeds of healthy English Morello pollinated by 4 different pollen donors

	Is–5	CR 6–29	CR 3–25	15–1–2
Virus status of pollen donor	Healthy	NRSV+PDV	NRSV+PDV	NRSV+PDV
No. seeds set/no. flowers pollinated	59/324	38/326	75/434	33/347
No. seeds germinated	45	30	37	21
No. seedlings with:				
PDV	0	2	1	7
NRSV	0	1	7	0
PDV+NRSV	0	1	2	1
Total seedlings infected	0	4	10	8

(b) *In the field.* A second series of crosses of virus-free English Morello females pollinated by virus-infected Montmorency was

Table 2. Virus transmission and associated data expressed as percentages. Seeds of healthy English Morello pollinated by 4 different pollen donors

	Is–5	CR 6–29	CR 3-25	15–1–2
Virus status of pollen donor	Healthy	NRSV+PDV	NRSV+PDV	NRSV+PDV
No. flowers pollinated	324	326	434	347
Per cent seed set.....	18.2	11.7	17.3	9.5
		Mean = 13.2		
Per cent germination	76.4	78.9	49.3	63.6
		Mean = 60.3		
Per cent seedlings infected.	0.0	13.3	27.0	38.1
		Mean = 25.0		

made in the field in May 1959. Techniques of pollination, seed germination, and seedling indexing were identical with those previously outlined. One of the English Morello female parents, originally virus-free, had evidently contracted NRSV during the 1958 season, and, when indexed just prior to pollination, was found to be infected. This tree was pollinated with pollen carrying NRSV and PDV and data was taken on the resulting seedlings.

Data from this experiment are presented in Table 3. As can be seen, these data conform rather closely to comparable data from the greenhouse experiments.

Table 3. Pollen transmission of NRSV and PDV to seeds of English Morello in the field

	Virus-free x Virus-free	Virus-free x Infected	Infected x Infected
No. seeds set/no. flowers pollinated	394/1459	649/3502	167/2857
Per cent seed set.....	27.0	18.5	5.9
Per cent seed germinated.....	21.8	21.3	38.3
Per cent seed infected.....	0.0	22.5	57.8[1]

1. Open-pollinated seeds of this female parent were 45.2 per cent infected.

(c) Sweet cherries (*Prunus avium* L.). Extensive experimentation with crosses of virus-free by virus-infected sweet cherries have not yet been undertaken. Random sampling of young sweet cherry seedlings resulting from a controlled cross of a virus-free Emperor Francis female pollinated by a virus-infected Schmidt's Bigarreau revealed that at least 4 seedlings of the 27 sampled were infected with NRSV. These data, while limited in scope, indicate that NRSV is pollen-transmitted in sweet cherry as well as sour cherry.

Conclusion

NRSV and PDV are of common occurrence and widespread distribution in cherries in the United States. Both viruses, if they spread at all in the nursery after propagation, do so in very rare instances, and in the eastern United States, young virus-free trees in the orchard seldom contract either virus prior to the 5th or 6th year after planting. During the period 7-10 years after planting, however, both viruses spread in the orchard at a rapid rate, so that 95 per cent or more of previously healthy trees commonly are infected in an orchard 10 years of age. A virus-free tree in the 15-20 year age class is a rarity. In spite of the numerous transmission tests with insects and mites carried out by several workers, no means of natural transmission of either virus in the orchard has yet been experimentally demonstrated.

The data presented here planly demonstrate that NRSV and PDV, either alone or in combination, are carried in pollen, that such infected pollen is capable of fertilizing ovules produced on virus-free trees, and that a relatively high percentage of the seeds resulting – 20-25 per cent – produce infected seedlings. Thus, pollen transmission of these viruses from tree to tree in the orchard seems a plausible hypothesis, for there appears to be no *a priori* reason why these viruses should not be translocated out of the developing fruits back into the mother tree. It is well to emphasize, however, that there is no evidence in our experiments that the maternal parent is infected by this means. All of the 55 virus-free female parents in our experiments have remained virus-free.

Whether or not tree-to-tree transmission of NRSV and PDV in

the orchard is by the avenue of pollination, pollen transmission of these viruses to seeds is of great economic importance in its own right. The principal means of distribution of these viruses is in infected nursery trees, which have become infected because they were propagated with infected materials. Even though many U.S. nurseries now propagate cherry nursery trees from virus-free scion sources, some 5-15 per cent of the young trees are already infected as they leave the nursery because of infections contracted from the seedling rootstocks on which they are propagated. Such infected nursery trees are not only unprofitable in the orchard but serve as sources of virus contamination for the remaining trees in the planting.

Previous work at this station has shown that commercially available mahaleb seedlings (*Prunus mahaleb*) commonly carry a virus incidence of 6-10 per cent, and, in occasional lots, nearly 20 per cent of the seedlings may be virus carriers. In recent years, a concerted effort has been made by American seed producers to rogue out infected mahaleb seed source trees and thus eliminate seedling infection. Pollen transmission of NRSV and PDV complicates this program tremendously, since there is now no assurance that seeds produced on a virus-free tree are, in fact, virus-free; infection may have been contracted from the pollen parent.

Similarly, in a breeding program designed to develop new cherry varieties, care must be taken to ensure that both parents are virus-free. Certain crosses may no longer be feasible, because virus-free trees of a given variety may not be available.

DISCUSSION

Gualaccini: Did every part of trees pollinated with infected pollen become infected, or did the virus only pass into the seeds on such trees?

Brase: Thus far the 55 virus-free female parent plants have remained virus-free, and the virus has only passed on to some of the seeds and seedlings.

Millikan: Are your results of Prunus cerasus comparable to the usual P. mahaleb, our commonly used seed source?

Do you consider the lower germination power noticed in your observations significant?

Brase: The answer to the first question is yes – that mean for the three virus sources used was 25 per cent. Twenty five per cent of the

seedlings obtained indexed positive for necrotic ringspot and prune dwarf virus.

According to tests carried out commercially available mahaleb seedlings (Prunus mahaleb) carry a virus incidence of 6-10 per cent and, in occasional lots nearly 20 per cent of the seedlings may be virus carriers.

In hand pollination normally a much lower fruit set is obtained than under normal conditions even with healthy pollen.

In every case a still greater reduction in fruit set occurred when pollen from virus carrying sources was used.

CROPLEY: It is most interesting that healthy trees pollinated with infected pollen set few fruits compared with those pollinated with virus-free pollen. This may be of importance for geneticists when studying pollen-ovule incompatibility.

Also it is of importance in variety cropping trials if the pollinator varieties are infected.

LUCKWILL: If one has an infected mother tree and pollinates it with pollen from a virus-free tree, does the virus then pass into the seed?

BRASE: Tests have shown that in such a case the presence of virus in some but not all of the seedlings, has been detected by indexing to an indicator plant.

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The effect of latent virus infection on the growth and yield of sweet cherry trees

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The viruses calibrated

Tests for virus infection have shown that most sweet cherry trees (*Prunus avium*), except those propagated from specially selected scionwood, in North Amerika and England are infected with viruses of the necrotic ring spot group. These viruses cause necrosis of the leaves of most varieties of sweet cherry in the first or second year of infection, but then the plants usually become symptomless. Trees propagated from scions taken from infected trees do not develop symptoms unless the virus is one of the "recurrent" strains from the effects of which trees do not fully recover.

Viruses of the necrotic ring spot group, in which sour cherry yellows is included, have long been known to cause a reduction in the growth and crop and Montmorency and other sour cherry (*P. cerasus*) varieties, on which the development of fruiting spurs may be prevented and general growth is reduced. Sweet cherry trees carrying latent ring spot viruses usually appear normal, however, and most growers would not think that they were suffering any loss of crop.

Both strains of necrotic ring spot viruses used in these trials produced similar necrotic symptoms on peach. Shirofugen (*P. serrulata*), and cucumber. One strain produced shock symptoms of tatter-leaf on *P. avium* and *P. cerasus* varieties, followed by recovery in the second year after inoculation and no symptoms subsequently except yellows on Montmorency. The second strain caused leaf necrosis in lines (necrotic line pattern) on the leaves

of all varieties inoculated, and these symptoms recurred undeminished in each subsequent year. Yellows symptoms developed on Montmorency.

Ring mottle virus is distinguished from necrotic ring spot by the absence of symptoms on peach, Shirofugen and cucumber; it causes ring mottle on the leaves of mazzard F12/1 and occasionally on Early Rivers.

The virus which causes rusty mottle on mazzard F12/1 and some commercial scion varieties of *P. avium* frequently occurs latent in other varieties particularly Napoleon and Turkey Heart. This virus seems to be distinct from those causing rusty mottle and necrotic rusty mottle in North America, and the prefix "European" is therefore used.

European rugose mosaic also differs from American rugose mosaic in that the former seldom causes leaf enations and produces less severe effects on Bing and Napoleon. Trees of Early Rivers, Napoleon, Turkey Heart and Merton Heart developed no symptoms, or only a transient leaf mottle when infected with rugose mosaic virus which caused recurrent mottling and leaf distortion on Merton Bigarreau.

Design of Experiments

Four experiments were done to assess the effects of these viruses. In the first, 6 mazzard F12/1 plants were inoculated with each virus when two years old, and their growth measured for 4 years. The trees were then cut at ground level and weighed. A second experiment was similar except that only ring mottle and rusty mottle were investigated, 72 F12/1 plants being inoculated with the former and 99 with the latter virus. These trials have already been described (Posnette & Cropley, 1956), and as the F12/1, trees were visibly affected the results are not included here with those obtained with other varieties in which the viruses were latent.

In the third experiment four commercial varieties were used to estimate the relative economic importance of the viruses commonly propagated in cherry trees sold by nurserymen. To avoid possible differences between types of the same cherry variety, all

the trees of each variety were propagated from a single virus-tested tree, using graded rootstocks of the mazzard clone F12/1. The trees were inoculated by patch grafting when 1 year old, using a single source of each virus, and planted (1953) with healthy controls at 12×15 ft. spacing in a randomised plot layout with twelve replications.

In the fourth experiment, 28-year-old trees were inoculated (1953) to assess the effect of the viruses on the fruit of mature trees, thus eliminating the indirect influence of reduced growth, and reproducing the effect of the viruses spreading in commercial orchards. 8 trees of Early Rivers and 8 of Turkey Heart were inoculated with each virus, the same number being used as uninoculated controls. These trees were indexed before inoculation; the Early Rivers trees were found to be already infected with ring mottle virus and the Turkey Heart trees with rusty mottle. The yields of each tree in previous years were known and comparable trees were chosen for the experiment, the treatments being randomised.

Virus spread

Long-term field trials to assess virus effects are liable to be invalidated by spread of the viruses to the controls. Previous tests had shown that the viruses causing ring mottle, rusty mottle and rugose mosaic had not spread appreciably from one variety to another, but it was known that the necrotic ring spot viruses had spread more rapidly in other orchards under observation. Records of symptoms developing on each tree were therefore taken every year.

In the trial with mature trees, 18 trees in the orchard became naturally infected by spread of the necrotic ring spot viruses from inoculated trees, but only 3 of these infections were in experimental trees, as the trial included only 55 of a total of 390 trees.

In the trial with young trees, tatter leaf developed in 1957 on 2 trees inoculated with rugose mosaic virus, and in 1959 on one inoculated with rusty mottle. Symptoms of the recurrent strain of necrotic ring spot developed (in 1956 and 1959) on two trees inoculated with the non-recurrent strain.

The amount of virus spread during the period of these trials was thus insufficient to influence the result at the level of replication adopted.

Trial with young trees

The girth of each tree was measured annually at 2 ft. above ground level. After six years, the healthy control trees of Early Rivers, Napoleon and Merton Bigarreau were about 10 per cent larger than the trees infected with rusty mottle and rugose mosaic and about 20 per cent larger than those infected with necrotic ring spot – sour cherry yellows. The slight reduction in growth of the ring mottle-inoculated trees was not significant. The fourth variety, Merton Heart was stunted 20 per cent by necrotic ring spot but not by the other viruses.

The viruses also reduced the yield of fruit. Ring mottle virus in Early Rivers, rugose mosaic and rusty mottle in Early Rivers, Napoleon and Merton Bigarreau reduced the crop by 20-30 per cent while the necrotic ring spot viruses did so by 79 per cent; the latter effect was considerably greater than the reduction in growth caused by these ring spot viruses.

Trees of Merton Heart, a low-cropping variety, reacted differently. Only the recurrent necrotic ring spot virus (necrotic line pattern) reduced the yield of fruit; ring mottle had no effect, but rusty mottle increased the yield by 50 per cent, while rugose mosaic and non-recurrent necrotic ring spot more than doubled it.

Tabel 1. Young trees: Weight of fruit 1957-59
as percentages of controls

	Control		Ring mottle	Rusty mottle	Rugose mosaic	Nec. ring spot		Sig. Diff. (P=0.05)
						Non-rec.	Recurrent	
	lb per tree	%	%	%	%	%	%	
Early Rivers.....	26.4	100	62.5	72.3	77.7	31.8	27.7	11.7
Merton Bigarreau	39.7	100	105.5	82.6	68.0	28.5	33.5	15.4
Napoleon.....	13.4	100	108.9	75.4	56.7	23.1	28.4	26.1
Merton Heart....	9.7	100	102.1	167.0	252.6	219.6	45.4	24.5

Trial with mature trees

The greater effect of the viruses on the yield than on the growth of trees was confirmed by the results with mature trees, in which the growth made during the 5-year period of the trial would be only a small fraction of the total size.

It is necessary to emphasize that the controls in this trial were not virus-free; the Early Rivers trees were infected with ring mottle virus, which reduced the crop of the young Early Rivers trees by nearly 40 per cent, and the Turkey Heart trees with rusty mottle virus which produced 20 per cent effect on young trees of three varieties. The addition of rusty mottle virus or of rugose mosaic to the Early Rivers trees and of the rugose mosaic virus to the Turkey Heart trees had little effect on yield compared with the already infected control trees. Both strains of necrotic ring spot virus, however, seriously reduced the yield of fruit. In the fourth, fifth and sixth years after inoculation the latent non-recurrent ring spot virus halved the crop of Early Rivers fruit, and that of the Turkey Hearts by 37 per cent. This virus has spread rapidly in orchards under observation since 1949, and trees in the shock "tatter-leaf" stage are frequently encountered during orchard surveys.

Table 2. Mature trees: Weight of fruit (lb.) per tree 1957-59

	Control	Rusty mottle	Rugose mosaic	Nec. ring spot/SCY		Sig. Diff.
				Non recurrent	Recurrent	(P=0.05)
Early Rivers	341.0	333.1	273.7	165.6	95.7	82.4
Turkey Heart	294.6	—	242.1	186.9	79.6	67.4

Virus effects on Merton Heart trees

The yields of the Merton Heart trees leave no doubt that infection with rusty mottle, rugose mosaic and non-recurrent necrotic ring spot resulted in an increase in fruit production, not accompanied by an increase in growth. These three viruses had less effect on the growth of Merton Heart trees than on that of the other varieties. It is clear that Merton Heart trees do not respond

in this way to all viruses; ring mottle virus had no effect on either growth or crop, while the recurrent strain of necrotic ring spot virus severely reduced both.

A plausible explanation would be an interaction between the viruses inoculated and a hypothetical latent virus in the apparently healthy clone of Merton Heart. No virus has been detected, however, by indexing on peach, Shirofugen and F12/1, and the only evidence of virus infection is a slight splitting and roughening of the Merton Heart bark similar to, but less severe than the rough bark virus canker described on the variety Frogmore (Posnette and Cropley, 1957). Inoculating Frogmore trees with scions of Merton Heart has not, however, induced any symptoms on the former. No trees of Merton Heart have been found entirely free from this rough bark condition, including those propagated from scions obtained direct from the John Innes Research Institute where the variety originated.

The most vigorous trees are the worst affected by rough bark, and slow-growing trees on a dwarfing rootstock have smooth bark. If the rough bark condition reduces the yield of fruit, a virus that slows the growth of the trees might thereby increase yield. If rough bark is a symptom of an undetected virus, the inoculascions of rusty mottle, rugose mosaic and non-recurrent ring spot may have also contained a virus that interferes with the multiplication of the rough bark virus and thus reduces its effect on yield.

Further experiments are in progress to test the Merton Heart clone for the presence of a virus that may be causing the rough bark, and efforts will also be made to eliminate the hypothetical virus by heat-treatment. Until such a virus is detected, we must conclude that although virus infection in general reduces the yield of most varieties, certain viruses have a beneficial effect on Merton Heart and perhaps on some other varieties not yet tested.

DISCUSSION

Nyland: Would tree decline increase with age in infected trees more than healthy trees (var. Merton Heart)?

Posnette: Not so far - infected trees are growing faster than before.

Jordovic: What about the quality of the fruits from infected trees?

Posnette: We have not noticed any change in the quality of the fruit, although trees infected with certain of the viruses ripen their fruit later.

Another effect of the viruses, especially rusty mottle, is to delay flowering so that pollination may be affected.

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The relationship of stone fruit ringspot virus to sour cherry yellows, prune dwarf and peach stunt

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Peach ringspot (3) or sour cherry necrotic ringspot (9) is a very common latent virus in many varieties and species of *Prunus*. The virus frequently produces a shock reaction upon first infection of many of its host plants which then recover and carry the virus without symptoms. The virus has probably become world-wide in its distribution (6). In the Handbook "Virus diseases and other disorders with virus-like symptoms of stone fruits in North America" (1) this virus is considered to be part of the complex described as Peach ringspot (pp. 71-80), Sour cherry necrotic ringspot (pp. 164-170), Sour cherry yellows (pp. 152-158), Prune dwarf (pp. 171-174) and Tatter leaf (pp. 141-146). During the past 10 years, several hundred trees have been indexed for ringspot virus. In no instance where adequate tests have been made has any trees been found free of the ringspot virus and still showing symptoms of any of the diseases listed above. Therefore, either these diseases are caused by strains of the ringspot virus or ringspot is always present as a contaminant.

When virus infected buds are placed in Shiro-fugen flowering cherry (*P. serrulata*) a necrotic reaction occurs around the area of bud insertion (5). This reaction has been generally accepted as a reaction of the ringspot virus, although trees showing Sour cherry yellows, Prune dwarf or Peach stunt will also give the reaction. This has been explained by the Shiro-fugen reaction being due to ringspot virus which is present in all cultures of Sour cherry yellows and Prune dwarf. Attempts to separate 2 viruses from the complex by differential tree fruit hosts have not been successful.

A virus was isolated mechanically by juice transfer from this complex to cucumber (2), and later the host range of the virus or viruses was extended to a number of herbaceous species (4). The use of squash varieties as differential hosts of viruses of this complex was reported in 1956 (7). Further studies with the squash varieties White Scallop (*Curcurbita Pepo* Linn.) and Buttercup (*C. maxima* Duchesene) have demonstrated that several strains of virus can be isolated and recognized from this complex of diseases. When viruses from several sources were isolated on squash they could be divided into two distinct groups. If the source trees indexed positive on Shiro-fugen, but were not infected with the virus which caused Prune dwarf, Peach stunt or Sour cherry yellows, the reaction was the typical necrotic non-yellows type of symptom on squash. All the other isolates caused a different reaction on the two squash varieties, which placed them in the squash yellows group.

This was demonstrated by the following experiment. A virus was isolated from each of 12 different stone fruit trees which had indexed positive on Shirofugen but did not index positive for Sour cherry yellows, Prune dwarf or Peach stunt on their respective index hosts. These 12 isolates caused only necrotic local lesions, or chlorotic areas, or rings on the cotyledons of Buttercup squash. The virus seldom became systemic, but when it did, localized scattered necrotic spots or areas were the only effects noted. A more severe reaction occurred on White Scallop squash. Local necrotic or small chlorotic spots or rings appeared on the inoculated cotyledons in 4 to 5 days. The more severe strains moved rapidly into the growing tip and killed it before any secondary leaves were formed. Some plants remained alive for two or more months without any secondary growth, but on others the buds produced dwarfed plants with small distorted, rosetted leaves. The milder strains were more difficult to transmit and often caused only occasional localized chlorotic areas on the large expanded secondary leaves. Occasionally one of these mild strains did become systemic and caused a severe distortion and misshapening of the leaves. These isolates became very difficult to transmit to squash after the month of April.

A virus was also isolated from each of 16 different stone fruit

trees which had indexed positive on Shiro-fugen and also positive for Sour cherry yellows, Prune dwarf and Peach stunt on their respective index hosts. Some of these isolates caused a few necrotic or chlorotic local lesion on the cotyledon leaves of Buttercup squash, but they all became systemic and usually caused a brilliant golden chlorotic mottle on the first leaf formed. As the new growth developed, some strains continued to cause a prominent golden chlorosis on all leaves while others caused only a mild golden fleck mottle or an occasional golden spot or golden area on the vein. Some isolates caused a severe dwarfing and distortion of the leaves. Local lesions were seldom produced on White Scallop squash, but the secondary growth was usually dwarfed, the leaves small and chlorotic without a definite pattern. There was no necrosis. Some isolates did not cause extreme dwarfing, and the plants and leaves were nearly normal in size. These leaves showed only a mild vein or veinlet mottle or at times a brilliant golden chlorosis of the midrib.

In another experiment, a collection of budwood was made from Italian prune trees from 40 different localities or orchards in the states of Idaho, Oregon and Washington. All showed normal foliage, but all indexed positive for ringspot virus on Shiro-fugen. A virus was isolated from each tree and transferred to White Scallop squash. None of these 40 virus isolates produced symptoms on White Scallop squash which were similar to the yellows type of reaction associated with isolates from Prune dwarf trees. This demonstrated the value of squash in identifying the various strains of ringspot virus associated with symptoms on stone fruit varieties.

Four virus isolates from the normal leaf types and 10 virus isolates from the yellows-prune dwarf types have been returned to tree fruit varieties. The inoculum was taken from young squash plants and transferred to young *P. mahaleb* seedlings in the 4 to 6 leaf stage. The young unfolding leaves were gently rubbed with infected juice, phosphate buffer and carborundum. The isolates from the normal-leaf trees were difficult to transfer back to mahaleb seedlings, and only 1 plant out of every 10 or more inoculated became infected. The isolates from the yellow-prune dwarf trees were readily transmitted to mahalebs. When buds

were taken from these inoculated mahalebs and placed in Shiro-fugen, all 14 gave a necrotic reaction on Shiro-fugen regardless of the source of the virus. All 14 isolates have also given a necrotic or chlorotic shock reaction when budded into virus free Montmorency sour cherry. The isolates from the yellows-prune dwarf group usually gave a chlorotic reaction on Montmorency but some gave a typical necrotic ringspot reaction or a mixture of chlorotic and necrotic ringspots.

In 1957, 7 isolates with a past history of sour cherry yellows, Prune dwarf and Peach stunt were transferred from squash to *P. mahaleb* seedlings by mechanical transfer of infected juice. The inoculated *P. mahaleb* seedlings which indexed positive on Shiro-fugen were then used as a source of infected buds to inoculate Early Muir peach, Montmorency sour cherry and Italian prune. All Early Muir peach trees inoculated with these 7 isolates developed severe peach stunt the next season after fall inoculation. The stunt symptoms have persisted after 3 years. All the Montmorency trees inoculated with the 7 isolates developed a chlorotic spot or ring mottle, and some were mixed with necrotic ringspots. Infected branches recovered after the shock reaction and the foliage appeared normal. In 1959, 6 of the 7 isolates caused Sour cherry yellows symptoms on some of the leaves. These trees are still growing very vigorously and do not show the severe Sour cherry leaf fall of the trees inoculated with buds from trees which have not gone through squash and mechanical transmission. The seventh isolate apparently moves very slowly through the tree and several branches still show the chlorotic shock reaction in 1960, and may eventually develop sour cherry yellows. All 7 isolates produced prune dwarf on Italian prune, with 4 isolates moving very rapidly through the tree, while the other 3 isolates have infected only a few branches close to the point of inoculation. Those branches showing prune dwarf symptoms index positive for virus on Shiro-fugen, but branches with normal leaves tested negative for virus. Apparently, only one virus was involved in causing prune dwarf and it gave a necrotic reaction for ringspot virus on Shiro-fugen.

Antagonism between the normal leaf types and prune dwarf types

Five different prune trees were observed to have individual branches bearing some leaf spurs with normal leaves and some with the narrow rough leaves of prune dwarf. When individual buds were taken from various branches on these trees and placed in peach seedlings, the two distinct types of virus were isolated from each of the five prune trees. Some of the inoculated peach trees were infected with strains that gave the necrotic reaction on squash, while others were infected with the non-necrotic, golden chlorosis type. Some peach trees were infected with both types. In general, the branches with prune dwarf symptoms gave the yellows types and the others gave the necrotic type reaction. Apparently the 2 virus types were antagonistic to each other in the prune trees and the strain which became established first in the branch was the predominating strain.

This same type of antagonism was noted in Early Muir peach inoculated with a peach stunt isolate from mechanically inoculated mahaleb. Three trees were inoculated and all developed a severe stunt reaction the following year. The branches were still stunted and rosetted the second year, except for one branch on one tree which grew normally and produced five feet of growth with normal leaves. When a virus was isolated from the tip leaves of this branch, it gave the normal leaf type of reaction on White Scallop squash, while the dwarf branches still gave the reaction for the yellow dwarf type. The origin of this strain of virus would be very interesting. Was it present as a contaminant in the original inoculum, or did a virus mutation occur in which the yellows-prune dwarf strain changed to the normal leaf strain? Regardless or origin, this would have to be interrupted as antagonism between two strains of the same virus.

Antagonism and cross protection between the two groups of isolates in Montmorency sour cherry. When virus free Montmorency trees are inoculated with Peach ringspot or Sour cherry necrotic ringspot, a shock reaction occurs and the trees then recover and new leaves are symptomless (1). When recovered trees are propagated with ringspot infected buds, no further shock

reaction occurs when reinoculated with ringspot virus. This information was used as a basis of testing antagonism or cross protection between the non-yellows and the yellows group of ringspot strains isolated in these studies.

Virus free Montmorency trees were inoculated with 7 strains of the mechanically transmitted virus of the yellows group. These viruses were given 2 growing seasons to become established in the Montmorency trees. Dormant scion wood was then taken from these trees and cleft-grafted onto dormant *P. avium* seedlings. At the same time, 2 chip-buds from a Montmorency infected with a severe strain of non-yellows ringspot were placed immediately below the Montmorency grafts. Six trees were grafted with virus free Montmorency scions and inoculated with the same strain of ringspot for checks. Four of these 6 virus-free Montmorency grafts grew and the foliage of all 4 developed a severe necrotic ringspot shock reaction. Thirty of the 42 grafts infected with the yellows group of ringspot strains grew and only 2 of these developed a necrotic ringspot reaction.

The experiment was repeated, and 14 virus-free trees and 9 trees from each of the 7 yellows infected source trees were prepared and inoculated as in the previous experiment. All of the 14 virus-free trees and 47 of the 63 virus infected grafts produced trees. Eleven of the 14 trees from virus free origin developed a severe necrotic ringspot shock reaction, and only 14 of the 47 trees from virus infected trees showed necrotic ringspot shock.

A summary of the two experiments is given in the following table:

Antagonism between yellows and non-yellows strains of ringspot

Strain of virus	Number of trees	Number with shock	Percent shock
RS 10	12	0	0
RS 11	10	0	0
RS 31	12	1	8.3
RS 29	12	2	16.6
RS 37	12	2	16.6
RS 23	7	3	42.8
RS 44	13	8	61.5
Checks	18	15	82.2

The degree of protection varied greatly between the 7 strains of virus from nearly complete protection in the first 5 strains to less than 50 per cent in the other 2 strains. Since ringspot virus usually moves rather rapidly through Montmorency, a two-year incubation period was assumed to be sufficient for the virus to move completely through the four-year old trees used in this experiment. The individual branches used for scion wood were not indexed for ringspot and some of them could have been free from virus. Several branches of the trees inoculated with strain RS 44 showed shock reaction when new growth developed the following season, indicating that the virus was still spreading in these trees. This could account for the lack of protection shown in some branches used in these antagonism studies.

CONCLUSIONS

All evidence obtained during these studies supports the theory that the stone fruit diseases known as Peach ringspot, Sour cherry necrotic ringspot, Sour cherry yellows, Peach stunt, Prune dwarf, and Cherry tatter leaf are caused by strains of the same virus. A recent report by Nyland (8) also includes Peach necrotic leaf-spot and Muir dwarf in this same group of diseases. The peach ringspot shock reaction, the sour cherry necrotic or chlorotic shock reaction, and the Shiro-fugen local necrotic reaction have been used by various workers as the test for the ringspot virus. All isolates used in these studies have fulfilled this definition. In addition to these reactions, some isolates also cause Sour cherry yellows, Prune dwarf, and Peach stunt after mechanical transfer to herbaceous hosts. The methods of virus transfer and the herbaceous hosts used should have separated any virus contaminants. The antagonism noted between the isolates in mature trees and the cross protection between 2 isolates noted in Montmorency sour cherry also indicates virus strain reactions rather than different virus entities.

DISCUSSION

Baumann: Did Dr. Milbrath transmit his non-yellows strain from squash to cucumber and what symptoms did he get on this host plant?

Milbrath: The non-yellows severe strains cause a sudden wilt and

collapse of plants 3-6 days after inoculation. Milder strains do not kill the cucumber but the plants do not develop the severe rosette of the yellow strains.

POSNETTE: At East Malling we have used Dr. Milbrath's varieties of squash and obtained similar symptoms. The yellow strains are associated with dark green rosette symptoms on cucumber.

SCHUCH: If a peach tree is infected with a mild strain of peach ring spot, is this tree then protected against peach stunt virus?

MILBRATH: The degree of protection or antagonism varies between strains.

I would expect complete protection between some strains and partial protection to no protection between others.

QUESTION: Are any hosts known which give similar symptoms with both yellows and non-yellows isolates? Comments are requested on the possibility that Shirofugen reacts similarly to more than one virus.

MILBRATH: Yes. -- In addition to the Shirofugen reaction all isolates cause a necrotic or chlorotic shock reaction on Montmorency sour cherry, and the peach ringspot reaction.

JORDOVIC: Have you always got positive reaction on Shirofugen using material infected with Prune dwarf virus?

MILBRATH: Yes.

GUALACCINI: What kind of reaction do you obtain when you insert on a cherry seedling a bud infected with ringspot virus below a Shirofugen bud?

MILBRATH: The Shirofugen dies as soon the buds begin to swell due to a necrosis at the graft line.

In America virus-free nursery stock grows much faster and the stand of buds in the nursery row may be as much as 50 per cent greater with virus-free buds.

Four year old virus-free trees of the variety Montmorency produce as much fruit as seven year old trees infected with ringspot virus.

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Morphological and anatomical changes of the fruits of some plum varieties infected by plum pox disease

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Most of the fruit viruses cause symptoms usually on the leaves without symptoms on fruits. Up to now only a few fruit virus diseases are known with distinct symptoms on the fruits. Among them plum pox attracted much attention because of the very characteristic and conspicuous effects on the fruits.

Among other investigations of plum pox virus disease in Yugoslavia, we studied varietal reaction to plum pox virus recently in order to find some resistant variety eventually. Some parts of these investigations will be the subject of this paper.

In general the main symptoms of plum pox disease on the fruits appear as sunken dots, lines and rings, with many crumplings. So the affected fruits have a pox appearance, what is the main reason for the name of the disease.

First symptoms on the immature fruits are mild; at this time only small sunken dots are seen on the surface of the fruits. During the ripening process the sunken areas are more pronounced, and when the fruit is ripe, they are definite. The symptoms are distributed all over the surface of the fruit, but they are concentrated usually near the stylar end. Sometimes, the symptoms are restricted to one side of the fruit only. In that case, the growth of the fruits is not uniform and fruits are misshapen.

On most varieties the symptoms are not limited to the surface of the fruit, but they extend in the flesh. Under the sunken parts of the fruit there is a change in the colour of the flesh to brown or reddish brown. Very often the tissue is necrotic with a bigger quantity of the gum. Also, the symptoms of the disease can be found on the stone of the infected fruits as dark round spots or rings.

The described symptoms are typical for the plum pox virus disease on the majority of the prune varieties, but there is some variation in symptom expression on different varieties. We found some morphological and anatomical changes which are different from those cited above. For example, Pearl and Green Gage do not show any morphological or anatomical changes on the fruits, but the infected fruits are smaller than healthy ones. Some other varieties as Bon de Brü, Ruth Gerstätter and Crvena ranka show small sunken dots concentrated near the stylar end. Under these dots can be found very small internal necrotic areas, which are localized under the skin only.

A second group of varieties such as Ersinger's Lombard and Washington shows symptoms in the form of intensive coloured lines and spots on the skin of the fruits. The colour of these blemishes is different from the normal colour of the fruits. Usually, fruits with yellow skin show reddish spots, and those with red skin show blue spots and lines. It seems that virus causes the alteration of the normal distribution of the antocyanins in the skin of the infected fruits. These varieties show very conspicious symptoms on the stone, appearing as rings or spots which can be used with certainty for diagnosis of the disease.

The majority of the other varieties investigated show more severe symptoms than those which we mentioned above. The most pronounced symptoms are on Cimer's plum, Pozegaca and Monarch. These varieties show severe changes in the mesocarp of the fruits. The colour of the tissue is changed from normal to reddish brown or dark brown, depending from the variety. For example, Monarch show very intensive red colour, like carmine, of the diseased tissue. Microscopical investigation shows that nearly all cells of the mesocarp contain red colour. Sometimes red cells are surrounded by normal cells, and red tissue extends like rays from the skin to the stone. In this tissue the gum is usually present and may be extremely abundant on some fruits. Very often many fruits can be found with gum pockets on the surface.

The effects of the morphological changes on the weight and size of the fruits of some varieties are shown in the table.

It can be seen that the diseased fruits are from 5 to 29 per cent

lighter and about 4-12 per cent smaller than normal ones. There is a decrease of the pulp weight in relation to the stone, too. On the basis of these investigations it can be concluded that there is varietal reaction to virus of plum pox and some varieties can tolerate the presence of the virus.

The weight and size differences between affected and healthy fruits are variable from season to season and depends on weather conditions. They are greater during a dry season.

In general, the varieties with free stones are more susceptible than those with cling stones. Also, the varieties which ripe earlier are more resistent than those which ripen later.

Mechanical Analysis of Fresh Fruits

Variety	Fruits	Weight of one fruit in gr.	Weight of the pulp in gr.	Percent pulp	Weight of the stone in gr.	Percent of stone	Size of the fruit in mm
De Montfort	healthy	30.4	28.98	95.32	1.42	4.68	40.6 × 36.5
	diseased	25.3	24.05	96.08	1.25	4.92	37.4 × 34.2
Bon de Brü	healthy	19.25	18.45	95.87	0.79	4.13	29.6 × 31.5
	diseased	17.13	16.29	94.27	0.84	5.73	28.5 × 30.05
Büler's prune	healthy	20.63	19.64	95.22	0.98	4.78	36.2 × 30.7
	diseased	19.55	18.56	94.93	0.99	5.07	34.8 × 30.4
Zimmer's Prune	healthy	21.25	20.01	94.16	1.24	5.84	36.0 × 30.8
	diseased	14.03	12.88	91.80	1.15	8.20	32.7 × 26.6
Washington	healthy	47.14	44.83	95.09	2.31	4.91	44.8 × 41.0
	diseased	46.32	45.02	97.19	1.30	2.81	41.7 × 40.6
Metlaš	healthy	14.29	13.41	93.84	0.88	6.16	29.9 × 26.2
	diseased	11.81	10.94	92.76	0.86	7.24	28.0 × 25.1
Diamond	healthy	42.68	40.68	95.31	2.00	4.69	39.2 × 38.1
	diseased	36.02	33.24	92.00	2.78	8.00	32.7 × 31.9
Monarch	healthy	35.00	32.70	93.42	2.30	6.58	42.9 × 34.9
	diseased	27.31	25.44	93.15	1.87	6.85	40.3 × 34.5
Pearl	healthy	34.20	32.50	95.02	1.70	4.98	41.5 × 35.4
	diseased	28.85	27.58	95.59	1.27	4.41	39.5 × 35.3
Imperial	healthy	38.10	36.32	95.32	1.78	4.68	48.2 × 35.1
	diseased	37.43	35.85	95.77	1.58	4.23	44.7 × 36.4
Agen	healthy	19.83	18.61	93.84	1.25	6.16	37.8 × 27.9
	diseased	15.37	14.12	91.86	1.25	8.14	34.5 × 26.2
Golden drop	healthy	34.81	32.54	93.48	2.27	6.52	43.3 × 35.1
	diseased	33.96	31.78	93.58	2.18	6.42	41.4 × 35.9
Grand duke	healthy	49.15	46.29	94.18	2.86	5.82	50.8 × 39.0
	diseased	31.45	29.19	92.81	2.26	7.19	43.4 × 33.8

There is no corelation between the intensity of symptoms on the leaves and fruits. For example, the variety Crvena ranka shows very distinct symptoms on the leaves and we use it very often as indicator plant, but the symptoms on the fruits are very mild.

DISCUSSION

Millikan: How much spread are you getting under field conditions? Did you obtain any foliage symptoms on sweet cherry?

Jordovic: After five years of investigation the percentage of infected trees in different regions varied from 3 to 70 (most commonly 30-70 per cent).

No plum pox symptoms occurred on the leaves of sweet cherry.

Baumann: In Bulgaria some varieties shows symptoms on the leaves only after infection with plum pox virus and the fruits on those trees are not affected. Is that the case in Yugoslavia also?

Jordovic: I found symptoms on the fruits of all the examined varieties, but some differences in symptom expression from one variety to another may occur. There seems to be no connections between the severity of symptoms occurring on fruit and those on the leaves.

Cropley: Does the virus produce symptoms on peach fruits?

Jordovic: No, but on the leaves.

Schuch: Peach seedlings are hostplants of the plum pox virus. In the year of infection the first symptoms consist of a clearing; later vein banding and chlorotic rings may appear.

Baumann: Is it certain now that plum pox virus is not seed-transmitted (which was stated by Christoff)?

Jordovic: I tried a few times to transmit plum pox by seed, but always with negative results.

Assay of the transmission of sarka virus disease by sap inoculation to herbaceous plants

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From the economic standpoint, the sarka virus disease (plum pox) is the most significant virus of fruit trees in Yugoslavia, hence a most detailed and planned study of this disease has been made in several of our scientific institutes. However, we made a special study of the possibilities of mechanical transmission of the sarka virus to herbaceous plants, with the purpose of discovering such a sensitive plant, which would serve as a suitable test plant in the investigation of the sarka problem. A certain number of authors have hitherto experimented with the mechanical transmission of virus from stone fruit trees to herbaceous plants. Moore et al. were the first who in 1948 succeeded in transferring the virus mechanically from the leaves of sour cherry to those of cucumbers. Hobbs (1951) succeeded in transferring mechanically the virus of necrotic ring spot from sour cherry to cucumber and pumpkin. Varney, and Moore (1951) studied the effect of different temperatures on the occurrence of symptoms on cucumbers which were infected with the virus from sour cherry. Milbrath (1953) succeeded in transmitting mechanically the virus from the petals of a cherry tree on to a cucumber. Weintraub and Willison (1953) and Willison and Weintraub (1953, 1954) considerably contributed to the method of inoculating the cucumbers with the virus from stone fruit trees. Boyle et al. (1954), in addition to cucumber, discovered the sensitivity of squash to the virus from stone fruit trees. Varney and Moore (1954) succeeded in transmitting mechanically the virus from some species of *Prunus* to tobacco and Zinnia. Heines (1956)

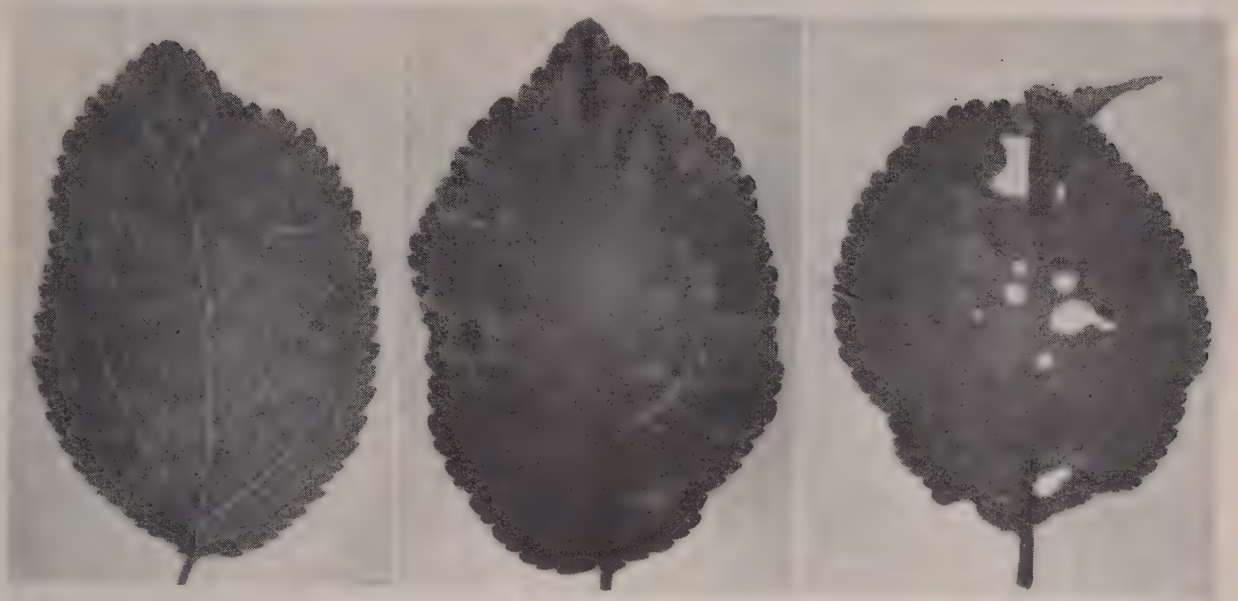

Fig. 1. Different symptoms of sarka on a leaf of a plum tree.
Left: chlorotic spots; in the middle: ring spots; right: vein banding and necrotic spots with holes.

transferred the virus to cucumber from 20 out of 23 cherry trees, which served as experiment plants, 22 of which were infected by the ring spot virus.

Considering the results and experience of the above mentioned authors, we began our tests of mechanical transmission of the plum sarka virus to different herbaceous host plants, whose results will be given here.

Material and Method

As a source of material for the preparation of the inoculum served the leaves from the diseased shoots of the Pozegaca plum, originally from an orchard in the vicinity of Zajecar, Berovo, and Cacak. The inoculum was prepared from the leaves of 32 Pozegaca shoots, which were grown in a glass-house. The following symptoms occurred on the leaves of these shoots: 1) chlorotic spots, 2) ring spots, 3) necrotic ring spots, with or without holes, and 4) line pattern (fig. 1).

For the preparation of the inoculum and the method of inoculation, we mainly applied the technique of Boyle et al. (1954) and Willison and Weintraub (1953, 1954). The inoculum was prepared by grinding the youngest diseased leaves from the shoots in a mortar in the presence of phosphatic buffer pH 6.98 and 7.17. For the grinding which lasted about 3 minutes, we used 1-5 grs. of leaves (most often 1 gr. only), and we carried this out in the presence of 3 parts of buffers. Thus, the obtained fresh sap served as inoculum, which we used directly with or without straining through a cloth. We conducted the inoculations of the leaves which were previously slightly sprinkled with carborundum powder, by smearing over them 5-6 drops of the inoculum with a glass inoculator. In one part of the test we rinsed the inoculated leaves, while in the other part this was not done.

The tests of inoculations were carried out from the beginning of May to the end of October. Out of 12 tests 4 were conducted in May when the diseased plum leaves used for making the inoculum were immature. The plants, which were tested as host plants, belonged to 25 different species and varieties of 8 plant families. The plants of the *Cucurbitaceae*-family were inoculated on the

Table 1. Plant Species tested as Host Plants of the Plum Sarka Virus

No.	Plant family	Plant species	Number of inoculated plants	Stage of growth when inoculated
1	Solanaceae	Nicotiana tabacum.....	15	
2		N. tabacum var. Jaka.....	15	
3		N. tabacum var. Virginia	153	up to 5 leaves
4		N. tabacum var. W. Barley...	80	
5		N. tabacum var. Ravnjak	91	
6		N. tabacum var. Samsun.....	25	
7		N. glutinosa.....	15	up to 7 leaves
8		N. silvestris.....	95	
9		N. rustica.....	95	up to 4 leaves
10		Lycopersicum esculentum....	15	
11		N. quadrivalvis	121	in different stages before flowering
12		Datura stramonium.....	40	cotyledons & first pair of secondary leaves
13	Cucurbitaceae	Cucumis sativus var. Delikates	150	cotyledons & secondary leaves
14		Cucurbita maxima.....	119	cotyledons with 1
15		Cucurbita pepo.....	151	& 2 secondary leaves
16		Citrullus vulgaris	152	cotyledons
17	Chenopodiaceae	Chenopodium amaranticolor .	35	up to 6 leaves
18		Chenopodium quinoa	35	in different stages of growth before flowering
19		Beta vulgaris var. saccharifera	40	2 & 7–8 leaves
20	Papilionaceae	Phaseolus vulgaris var. Cućavac.....	91	cotyledons & with 1 & 4–5 leaves
21	Compositae	Zinnia elegans	54	up to 4 leaves
22	Amarantaceae	Gomphrena globosa	11	
23	Caryophyllaceae	Melandrium album	45	First pair of secondary leaves
24	Rosaceae	Prunus armeniaca	15	seedling up to 15 leaves
25		Prunus domestica var. Crwena ranka	5	seedling up to 6 secondary leaves

primary leaves, while with the other families, this was done on the secondary leaves. The number of the inoculated plants and their stages of growth during the inoculation is shown in table 1.

THE RESULTS

The symptoms of the disease on *C. maxima* appeared in the inoculations made in May and June, and on 5 plants only. The basic symptom of the disease was manifested by general yellowing, which was followed by the drying off of the first pair of secondary leaves, including the top of the stem, later on. With one of these plants, round chlorotic spots were more clearly pronounced on the primary leaves, while on the first secondary leaf there was a vein clearing, which was later succeeded by vein necrosis. The first pair of secondary leaves and the top of the plant soon dried off and died (fig. 2). With the sap of this plant we caused the infection on *N. quadrivalvis*, on whose leaves the disease was manifested first in the shape of a slight mosaic, and later on as a general chlorosis of the whole plant.

Among *C. pepo* inoculated in the middle of May symptoms appeared in 3 plants.

The primary symptoms of the leaves are characterised by the occurrence of vein banding, whitish to pale yellow in colour (fig. 3). These symptoms then disappear, and instead of them a general chlorosis of leaves takes place. With the inoculations of the sap from one of these

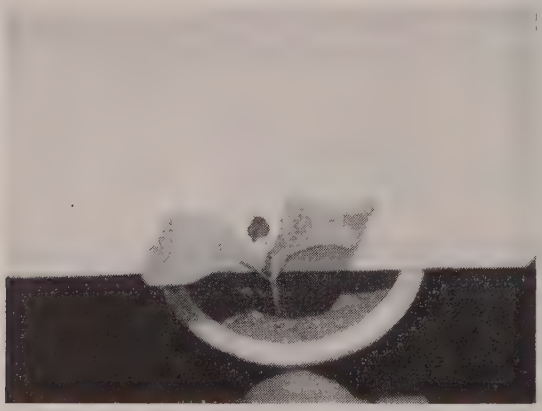

Fig. 2. Drying off of the first pair of secondary leaves of C. maxima, resulting after the inoculation of its cotyledons with the sarka virus

Fig. 3. Whitish and pale yellow zone on secondary leaves of *C. pepo*, resulting after the inoculation of its cotyledons with the sarka virus

plants we produced local lesions on the leaves of *C. maxima* and on the primary leaves of *Citrullus vulgaris*.

Among the *Solanaceae* plants the inoculations were positive on *N. quadrivalvis* only. The infections took place on 4 plants inoculated during the middle of May. All the later inoculations as well as the above mentioned species remained negative. With the infected plants there was a slight vein clearing on the young undeveloped leaves, while on the older ones there was chlorotic areas with general decoloration. In further re-inoculations the infected young plants soon died, while the older ones lagged behind in growth, became yellow, and dried off before the healthy plants (fig. 4).

DISCUSSION

The susceptibility of *C. maxima* and *C. pepo* to the viruses of stone fruit have been determined before by certain other authors. Thus, Boyle, Moore and Keitt (1954) determined, as stated before, the susceptibility of *C. maxima*, while Hobbs (1951), Weintraub, and Willison (1953) determined the susceptibility of *C. pepo* to these viruses.

Fig. 4. Mosaic and chlorotic leaves of *N. quadrivalvis*, resulting after the inoculation with the sarka virus

Whether these two species of Cucurbitaceae, according to the successful inoculations, present the sensitive host plants for the sarka plum virus, will be possible to assert after our one year old results have been further confirmed. In any case, the works of the above mentioned authors speak in favour of such possibilities.

The obtained positive results of the inoculations on *N. quadrivalvis* are interesting, since, as far as we know, this plant species has not been studied as a host plant for the viruses of stone fruit trees before. Considering the suitability it showed in these studies, the plant deserves to have special attention in our further work.

The above mentioned author Boyle with his collaborators, has stated that the age of leaves for the inoculum preparation has an effect on the mechanical transmission of the virus. The inoculations in our tests, which were successful mainly in May, when the infected plum tree leaves were immature, confirm the statements of the author mentioned above. Considering the significance of this, we will in our further work make special tests on the effect of age on the diseased leaves of plum trees, when making mechanical transmissions of the sarka virus.

CONCLUSION

In the assay of the mechanical transmission of sarka virus to different herbaceous host plants, the infections were manifested on *C. maxima, C. pepo,* and *N. quadrivalvis*. The results achieved in transmitting the sarka virus, cannot be considered definite as yet, since back transmission from the infected herbaceous plant to plum trees has not so far been obtained. Hence, we are continuing this work by proceeding with the isolation of new virus isolates, making a comparative study of their characteristics.

DISCUSSION

Milbrath: What species of squash did you use as testplant for Sarka disease?

Sutic: The variety Beogradsxa bela.

Schuch: Have you tested the source tree of sarka-virus for any other viruses?

In some trees I have found other viruses besides the sarka-virus such as ring spot and plum line pattern.

Sutic: So far mechanical sap transmissions have been carried out with inoculum from infected leaves taken from different shoots of the variety Pozegaca. In future experiments the source trees will be tested for other viruses.

Kegler: Were you able to transmit the sarka-virus right through the growing season or only during the spring months?

Sutic: Successfull sap transmission to herbaceous plants were only obtained during the spring.

Schmid: Did you make a second transmission from herbaceous plants to herbaceous plants? And if so, did you get more severe symptoms after the second transmission?

Sutic: A second transmission was performed but the symptoms were the same.

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Characteristics of a virus endemic in wild prunus

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A mechanically transmissible virus was found very commonly in wild *Prunus pennsylvanica*. These trees were being tested as possible sources of seed for experimental use, and most appeared healthy. The virus, designated H for convenience, was readily transmitted to cucumber, where it produced symptoms resembling those of necrotic ringspot. The experimental host range, however, differed from that of the necrotic ringspot virus commonly found in orchard trees.

Isolate H caused a severe systemic disease, often fatal, in *Vigna sinensis*, a species which could not be infected with previously described isolates of necrotic ringspot. Other hosts of isolate H which were not susceptible to viruses previously isolated from stone fruit were mainly legumes, in particular several *Phaseolus* species, *Soya max* and *Dolichos biflorus*. These host range differences suggested that isolate H might represent a virus distinct from necrotic ringspot.

On many other hosts isolate H produced symptoms much like those of necrotic ringspot or recurrent necrotic ringspot viruses. A number of hosts in which the latter two viruses had not become systemic, or had caused very mild symptoms, showed striking symptoms when infected with isolate H. Such hosts were *Helianthus annuus, Coleus blumei, Gomphrena globosa,* and *Zinnia elegans*. Local necrotic lesions were produced on *Momordica balsamina* and *Dolichos biflorus*. The latter host was used in most property determinations.

The physical properties of isolate H were very similar to those of necrotic ringspot virus. In undiluted cucumber sap infectivity was lost within a few minutes. Extracts in pH 8.0 phosphate

buffer representing 1:25 to 1:80 dilutions lost about ½ their infectivity each hour of standing at room temperature. The thermal inactivation point of the virus in 1:50 buffer extracts was 54°C.

It was found previously that the infectivity of necrotic ringspot virus in diluted cucumber extracts could be stabilized by including in the extract 0.01 M sodium diethyldithiocarbamate. Prune dwarf virus, on the other hand, required for stabilization the presence of a reducing agent such as 0.02 M sodium thioglycolate, in addition to carbamate. Isolate H behaved in this respect in the same way as necrotic ringspot virus in that infectivity was stabilized by carbamate. No reducing agent was required.

Cucumber cotyledons infected with isolate H provided more infectious inoculum 3 to 5 days after inoculation than did cotyledons infected for longer periods. Such inoculum was used to infect seedling *Prunus mahaleb* and *P. pennsylvanica*. The virus was then transmitted from these hosts by grafting to *P. cerasus* vars. Montmorency and English Morello. In these two hosts shock symptoms very similar to necrotic ringspot were caused.

P. pennsylvanica, after developing necrotic spotting as a result of infection by mechanical inoculation, produced leaves without symptoms, but which contained virus. When such recovered seedlings were reinoculated by grafting with known necrotic ringspot or recurrent necrotic ringspot viruses a second set of shock symptoms developed. It was sometimes necessary to graft-inoculate H-infected seedlings a second or third time to induce the second set of shock symptoms.

The behavior of isolate H in cross protection tests and host range studies indicated differences between it and necrotic ringspot virus. On the other hand, properties of the virus and symptoms induced in sour cherry were very similar to those of necrotic ringspot virus.

In attempts to identify isolate H more definitely is was purified by the same method as used previously for necrotic ringspot virus. Electron micrographs showed the virus as small spheres approximately the same size as necrotic ringspot virus – between 20 and 25 millimicrons.

Antiserum was prepared to necrotic ringspot virus and to isolate H by a series of intramuscular injections of purified virus emulsified in Freund adjuvant. Titers up to 1:1280 were obtained after 8 to 10 injections of rabbits at 4-day intervals. Isolate H reacted strongly with necrotic ringspot virus antiserum, and necrotic ringspot virus reacted strongly with isolate H antiserum. Neither of these two viruses reacted with prune dwarf virus antiserum. When virus antibody in isolate H antiserum was removed by absorption with necrotic ringspot virus the serum failed to react with virus H. From the results of serological tests it was concluded that isolate H was a strain of necrotic ringspot virus. It was also apparent that host range differences exist between strains of this virus. It was also evident that, with the methods used, cross protection tests had failed to indicate strain relationships.

In making surveys for incidence of virus infection in wild Prunus, isolate H was found in about 50 per cent of the *P. pennsylvanica* tested, and in smaller percentages of *P. serotina, P. virginiana,* and *P. americana.* It was the only mechanically transmissible virus found in several hundred wild Prunus trees tested. It was seed transmitted through 60 per cent or more of the seed of infected *P. pennsylvanica* trees.

On the other hand, less than half of the isolates of necrotic ringspot virus from commercial sour cherry orchards would infect cowpea or bean. The presence of isolate H in wild Prunus in areas far removed from cultivated plants, and the occurrence of young *P. pennsylvanica* showing shock symptoms indicated that this virus was endemic in wild Prunus. The presence of other strains in cultivated Prunus, but not in wild Prunus suggested that virus spread might be from wild to cultivated trees, but not in the reverse direction.

DISCUSSION

Milbrath: Have you placed other ringspot cultures in Chenopodium amaranticolor and if so what are the results?

Fulton: None others have infected C. amaranticolor, but this may be due to ineffective attempts. Necrotic ringspot virus (isolate G) will systemically infect C. album.

Millikan: Was there any cross-protection with recurrent ringspot?

FULTON: Necrotic ringspot and recurrent necrotic ringspot viruses reciprocally cross protected in Prunus pennsylvanica. Other isolates did not.

POSNETTE: Did you get cross-protection between isolates of ordinary necrotic ringspot using the same method of bud-inoculation?

FULTON: Yes, but the result may depend on the variety of the host plant.

I obtain cross-protection between recurrent and nonrecurrent strains of NRSV in Prunus mahaleb, but Dr. Moore does not in P. cerasus (Montmorency).

KUNZE: I have got systemic symptoms on Chenopodium amaranticolor like those demonstrated by Dr. Fulton after the infection with a latent virus from plum.

Erfahrungen über den Gebrauch krautiger Testpflanzen beim Nachweis von Steinobstviren

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Die mechanische Übertragung von Steinobstviren auf krautige Pflanzen gelang erstmalig im Jahre 1948 (Moore, Boyle & Keitt). Die Anwendung dieser Methode hat unsere Kenntnisse über Steinobstviren in den letzten Jahren ganz erheblich bereichert. Von den vielen Veröffentlichungen zu diesem Thema seien nur erwähnt die Arbeiten von Baumann 1956, Boyle, Moore & Keitt 1954, Fulton 1957a, b,1958, Gilmer 1957, Milbrath 1956, Mulder 1954, Weintraub & Willison 1953, 1954, Willison 1951, Willison & Weintraub 1953, 1954. In den U.S.A. wurden krautige Pflanzen auch zum Nachweis latenten Virusbefalls beim Steinobst eingesetzt (Boyle 1953, Gilmer, Brase & Parker 1957). In Europa verfügen wir jedoch auf diesem Gebiet erst über geringe Erfahrungen. Es wird deshalb hier über eigene Untersuchungen berichtet, die klären sollten, ob und in welchen Fällen z.Zt. krautige Testpflanzen beim Nachweis von Steinobstviren eingesetzt werden können. Da die Arbeiten ausschließlich praktischen Zielen dienten, handelt es sich dabei z.T. um Wiederholungen oder Abänderungen bekannter Versuche.

Als Infektionsmaterial für eine mechanische Übertragung auf krautige Pflanzen eignen sich am besten aufbrechende Knospen von Kirschen und sehr junge, noch gefaltete Blätter von Pfirsichen. Diesem Material wurde vor dem Zerreiben im Mörser etwas Sörensen-Phosphatpuffer (P_H6,5, 0,03 mol) zugesetzt[1], und zwar auf 10-12 Kirschenknospen etwa 1 ml, bei Pfirsichen etwas weniger. Als Testpflanzen dienten Gurken (Sorte Delika-

1. Nach *Cadman* (1959) ist ein P_H-Wert von 8,0 günstiger.

tess), Tabak (Samsun und White Burley) und *Chenopodium quinoa*. Wurden die Knospen von *treibenden* kranken Bäumen abgenommen, so zeigte sich in der Regel ein recht guter und gleichbleibender Infektionserfolg. Dagegen war das Ergebnis der Abreibung des öfteren negativ, wenn die Reiser virushaltiger Bäume bereits *während der Winterruhe* geschnitten und im Zimmer angetrieben wurden.

Eine direkte Virusübertragung von der Kirsche auf krautige Pflanzen läßt sich also mit gutem Erfolg nur während des Frühjahrs ausführen, und zwar nur innerhalb eines kurzen Zeitraumes. Deshalb wurde untersucht, wie lange man Reiser mit aufbrechenden Knospen bei kühlen Temperaturen aufbewahren kann, ohne daß die Sicherheit des Virusnachweises erheblich beeinträchtigt wird. Für diesen Versuch wurden Reiser von Vogelkirschen (*Prunus avium*) mit Stecklenberger- bzw. Ringflecken-Krankheit[1] als Virusspender und Gurken als Testpflanzen verwendet. Die Reiser wurden am 5.4.60 geschnitten und bei Temperaturen zwischen +2 und +5°C im Kühlschrank aufbewahrt. Wie die Tabelle 1 zeigt, brachte die mechanische Virusübertragung von Kirsche auf Gurke noch in der 2. Maihälfte recht gute Infek-

Tabelle 1. Infektionserfolg bei der mechanischen Übertragung von kühl aufbewahrten, virushaltigen Kirschenknospen auf Gurken

Infektionsdatum	Stecklenberger-Krankheit	Ringflecken-Krankheit Virusquelle		
		1	2	3
5.4.60[a]	8/10[b]	7/10	.	.
20.4.60.....	8/10	7/12	7/10	10/12
20.5.60.....	3/12	3/12	.	4/12
4.7.60.....	1/12	2/12	1/12	3/12

a. Frischgeschnittene Reiser.

b. Zähler: Zahl der erkrankten Gurken. Nenner: Zahl der abgeriebenen Gurken.

1. Vergleichende Untersuchungen zwischen den Erregern der Kirschen-Ringflecken-Krankheit in Mitteleuropa und den amerikanischen Viren der »Cherry ring spot«-Gruppe sind meines Wissens noch nicht durchgeführt worden. Es ist also möglich, dass die Namen »Kirschen-Ringflecken-Krankheit« und »Cherry (necrotic) ring spot disease« nicht genau das Gleiche bezeichnen.

Abb. 1. Rosettenwuchs an Gurke nach Infektion mit einem Virus der Kirschen-Ringflecken-Krankheit (Aufnahme: BBA Berlin Dahlem).

tionserfolge. Werden die Teste zu einem späteren Zeitpunkt angesetzt, so besteht u.a. die Gefahr, daß die Infektion der Gurken wegen zu hoher Gewächshaustemperaturen mißlingt.

Wie aus dem Vorangegangenen zu ersehen, eignet sich die Gurke für den Nachweis der Stecklenberger-Krankheit und der Kirschen-Ringflecken-Krankheit[1]. Auf die Erreger der beiden Virosen reagiert die Gurke nach 4-10 Tagen mit Flecken auf den abgerieben Keimblättern, mit Scheckung und Mißbildung der echten Blätter, z.T. auch mit Nekrosen des Blattgewebes und mit einer Hemmung des Triebwachstums (Rosettenwuchs, Abb. 1). Mitunter ist die Reaktion der Gurke so heftig, daß nur ein kleines nekrotisches, erstes echtes Blatt gebildet wird und die Pflanze nach kurzer Zeit welkt und zusammenbricht. Dies ist in der Regel der Fall, wenn mit der Stecklenberger-Krankheit gearbeitet wird.

1. Ob die Gurke auf alle Herkünfte der Kirschen-Ringflecken-Krankheit anspricht, sei dahingestellt, doch sprechen amerikanische Untersuchungen dafür (*Gilmer & Brase* 1956).

Das charakteristische Virus der Pfeffinger-Krankheit, das zu den Viren der Tabak-Ringflecken-Gruppe gehört (Kunze 1958, Pfaeltzer 1959), erzeugt dagegen auf Gurke nur sehr milde Symptome, und auch diese nicht so regelmäßig, wie es für einen Test erforderlich wäre. Außerdem können diese Krankheitszeichen der Gurke leicht durch die heftige Reaktion auf ein latentes Virus überdeckt werden, wenn in den untersuchten Kirschbäumen eine Mischinfektion vorliegt, was bei pfeffinger-kranken Bäumen häufig vorkommt. Als Testpflanze für die Pfeffinger-Krankheit kann deshalb die Gurke nicht eingesetzt werden.

Doch auch der Nachweis der Stecklenberger-Krankheit und der Kirschen-Ringflecken-Krankheit mit Gurke ist nicht immer einfach. Folgendes muß beachtet werden:

1. Die Gurke muß sich im richtigen Infektionsalter befinden. Die Keimblätter, die durch Pressaft-Verreibung infiziert werden, sollen zwar schon verhältnismäßig gut ausgebildet sein, das erste echte Blatt darf aber nicht länger als 3 mm sein. Für eine termingerechte Infektion steht also nur eine Zeitspanne von höchstens 2 Tagen zur Verfügung.
2. Die Gewächshaustemperatur soll nach Möglichkeit 25 °C nicht überschreiten, da sonst die Gurken nicht mit genügender Sicherheit reagieren. Die optimale Temperatur für die Symptomausbildung der Gurken liegt noch etwas tiefer.
3. Die Zahl der Gurken je Test darf nicht zu niedrig sein, da in der Regel nur ein Teil der abgeriebenen Gurken Symptome entwickelt.

Wieviel Gurken für jeden Test benötigt werden, wurde in einem Versuch geprüft, in dem je Probetest 4, 10 oder 20 Gurken verwendet wurden. Als Testobjekt dienten 2-jährige Sämlinge von *Prunus avium* und *Prunus persica,* die im vorangegangenen Jahr durch Schildchenpfropfung (chipbudding) mit 2 Herkünften der Kirschen-Ringflecken-Krankheit infiziert worden waren. Auf die eine Herkunft (a) sprach der Pfirsich als Testpflanze recht gut an, während die andere (b) auf Pfirsich gar keine oder nur sehr schwache Symptome hervorrief. Aus der Tabelle 2 ist zu ersehen, daß bei 18 von 22 inokulierten Vogelkirschen und bei 20 von 21 inokulierten Pfirsichsämlingen Virusbefall nachgewiesen werden konnte, wenn je Test 10 Gurken benutzt wurden. Der

Prozentsatz der Bäumchen mit Symptomausbildung schwankte dagegen zwischen 30 und 80 Prozent. Die Verwendung von 20 Gurken je Test brachte nur eine geringfügige Verbesserung der Ergebnisse. In den positiven Nachweisen bei Kirsche mit 10 Gurken je Test erkrankten in 3 Fällen nur eine von 10 Gurken, in 5 Fällen zwei und in den übrigen Testen jeweils vier oder mehr Gurken. Beim Pfirsich war das Ergebnis ungefähr ähnlich. Bei 20 positiven Nachweisen entwickelte in 3 Fällen nur eine von 10 Gurken Symptome, in einem weiteren Fall waren es zwei und in den übrigen Nachweisen vier und mehr Gurken je Test. Für praktische Zwecke scheint also die Zahl von 10 Gurken je Test ausreichend zu sein, um auf schnelle Weise beim Steinobst die Viren weitgehend zu erfassen, die mit Hilfe der Gurke nachweisbar sind. Da ohne Schwierigkeit 3 Gurken in einen 9-cm-Topf gepflanzt werden können, läßt sich allerdings die Zahl der Gurken je Test ohne weiteren Platzaufwand auf 12 erhöhen.

Tabelle 2. Erprobung des Virusnachweises mit Gurken bei experimentell infizierten 2-jährigen Steinobstsämlingen

Pfropfinfektion der Sämlinge: April-August 1959
Gurkentest: März-April 1960

Untersuchungsmaterial	Zahl der untersuchten Bäume	Bäume mit Symptomen (1959 oder 1960)	Bäume mit positiver Virusaktion auf Gurke		
			jeder Test mit 4 Gurken	jeder Test mit 10 Gurken	jeder Test mit 20 Gurken
Ringflecken-Krankheit Herkunft a					
Prunus avium	13	4	10	11	11
Prunus persica	15	12	9	14	15
Ringflecken-Krankheit Herkunft b					
Prunus avium	9	4	4	7	7
Prunus persica	6	3	6	6	6
Kontrollen (unbehandelt)					
Prunus avium	4	0	1	1	1
Prunus persica	5	0	0	0	0

Die Versuche mit der Virusherkunft »b« zeigten ferner, daß dieses Virus auch gut in Pfirsichen nachweisbar ist, die selber keine Anzeichen der Viruserkrankung ausbilden. Ein ähnliches Ergebnis lieferte auch eine andere Versuchsreihe, die im April 1959 mit dieser Herkunft der Ringflecken-Krankheit angesetzt worden war. Damals wurden durch Pfropfung 10 Pfirsich-Sämlinge infiziert, von denen nur 3 leichte Symptome entwickelten, und zwar erst im Frühjahr 1960. Trotzdem konnte bereits im Sommer 1959 bei 7 der infizierten Pfirsiche eine Virusinfektion durch mechanische Übertragung auf Gurke nachgewiesen werden. Wird der Pfirsich als Testpflanze für Kirschenvirosen verwendet, so können also zweifelhafte Reaktionen dieses Indikators schnell im Gurkentest überprüft werden.

Das Virus der Pfeffinger-Krankheit, das im Gurkentest nicht mit Sicherheit erfaßt werden kann, erzeugt auf Tabak ähnliche Symptome wie die Viren der Tabak-Ringflecken-Gruppe, in deren Verwandtschaftskreis es gehört. Etwa 6 Tage nach der Infektion entstehen auf den Abreibeblättern zahlreiche nekrotische Ringe. Unter geeigneten Bedingungen bilden sich später auf den Folgeblättern große helle, konzentrische Ringe und gewellte oder im Zick-Zack laufende hellgrüne Bänder, die feine Nekrosen aufweisen können. Diese systemische Erkrankung ist bei der Sorte »White Burley« meist stärker als bei »Samsun«-Tabak. Auch einige samenübertragbare Viren der Süßkirsche – vermutlich Stämme der Kirschen-Ringflecken-Krankheit – erzeugen auf Tabak Symptome. So konnte z.B. bei dem Test von 24 unbehandelten, 4-jährigen *Prunus avium*-Sämlingen durch die Pressaftübertragung auf Tabak viermal latenter Virusbefall nachgewiesen werden, durch eine gleichzeitige Abreibung auf Gurken dagegen nur zweimal. Die Symptome des Tabaks bestanden aus nekrotischen Ringen auf den Abreibeblättern und Linienmustern mit Nekrosen, größeren Flecken und einzelnen Ringen auf den Folgeblättern (Abb. 2). Sie besaßen also eine gewisse Ähnlichkeit mit den Symptomen der Tabak-Ringflecken-Krankheit. Leider eignet sich der Tabak aber nur wenig für den Nachweis von Steinobstviren, weil er ab Anfang April bereits recht unsicher auf diese Viren reagiert.

Ein sehr guter Indikator für die Pfeffinger-Krankheit ist dagegen *Chenopodium quinoa*. Diese Meldenart spricht außerordent-

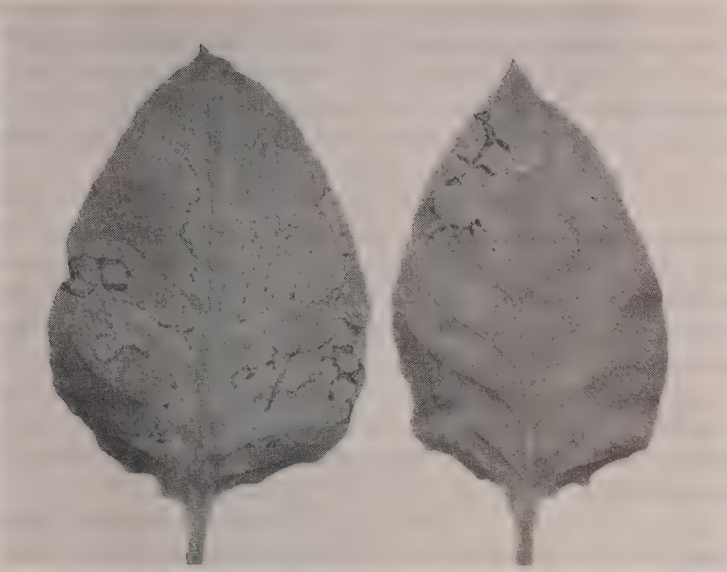

Abb. 2. Systemische Symptome an Samsun-Tabak nach Infektion mit einem Virus von latent befallenden *Prunus avium*-Sämlingen (Aufnahme: BBA Berlin-Dahlem).

lich sicher auf das Tabak-Ringflecken-Virus der Pfeffinger-Krankheit an. Die Pflanze bildet 5-8 Tage nach der Infektion graubraune, nekrotische Flecken auf den Abreibeblättern, wenig später zeigen auch die jüngeren Blätter Flecken und Ringe, die bald nekrotisch werden, und schon kurze Zeit darauf beginnt die Pflanze abzusterben. Da diese Reaktion mit großer Regelmäßigkeit auftritt, genügen für einen Test 2-3 Pflanzen. Als Indikator für das Virus der Pfeffinger-Krankheit ist *Chenopodium quinoa* empfindlicher als *Chenopodium amaranticolor*, das auf dieses Virus mit Lokalläsionen reagiert.

Dies zeigte sich in einer Testserie, die in diesem Frühjahr probeweise mit experimentell infizierten *Prunus avium*-Sämlingen durchgeführt wurde. Diese Pflanzen waren im Sommer 1959 mit kranken Rindenschildchen (chips) okuliert worden. Da ein Teil der Rindenschildchen nicht verwuchs, erkrankten einige der Bäumchen nur verhältnismäßig leicht. Bei allen Bäumen, ins-

gesamt 16, ließ sich bereits zur Zeit des Knospenausbruchs das Virus der Pfeffinger-Krankheit durch Übertragung auf *Chenopodium quinoa* nachweisen, u.a. auch bei einem Baum, der bis jetzt nur Symptome der Kirschen-Ringflecken-Krankheit zeigte. Im gleichzeitig durchgeführten Test mit jeweils 2 *Chenopodium amaranticolor*-Pflanzen war der Befund dagegen nur in 12 Fällen eindeutig positiv, in einem Fall fraglich positiv und in 3 Fällen negativ. Frühere Versuche hatten bereits gezeigt, daß *Chenopodium quinoa* auch dem Tabak als Testpflanze überlegen ist. Läßt man z.B. Tabaksaft, der dieses Virus enthält, *in vitro* altern, so kann man die Infektiosität des Virus im aufbewahrten Pressaft mit *Chenopodium quinoa* viel länger nachweisen als mit Tabak. Auch in Versuchen mit kühl aufbewahrten, virushaltigen Kirschenknospen zeigte sich bei *Chenopodium quinoa* als Testpflanze bis in den Frühsommer hinein kein Absinken des Infektionserfolges.

Außer dem Virus der Pfeffinger-Krankheit kann auch der Erreger der Stecklenberger-Krankheit auf *Chenopodium quinoa* Symptome hervorrufen. Die Erkrankung verläuft allerdings verhältnismäßig leicht, ihre Symptome erscheinen meist erst 10-12 Tage nach der Infektion. Sie bestehen aus kleinen, hellen Flecken und kurzen Bändern auf jüngeren, systemisch erkrankten Blättern, die etwas gekräuselt sein können. Die Flecken werden mit zunehmendem Alter der Pflanzen allmählich undeutlich. Vor kurzen berichteten Nemeth & Kegler (1960), daß bestimmte Herkünfe der Stecklenberger-Krankheit auch eine Nekrose der Triebspitze bewirken können. Da die Symptomausbildung aber nicht so regelmäßig erfolgt wie für einen Test notwendig, ist unter den krautigen Pflanzen die Gurke für den Nachweiss der Stecklenberger-Krankheit besser geeignet als *Chenopodium quinoa*.

Zusammenfassend läßt sich sagen: Auch krautige Pflanzen eignen sich für den Nachweis einheimischer Kirschenviren, wenn beim Test bestimmte Voraussetzungen beachtet werden. Da diese Viren in ihren krautigen Wirten nur eine kurze Inkubationszeit durchlaufen, können krautige Testpflanzen vor allem eingesetzt werden für eine schnelle, grobe Erfassung des Virusbesatzes bei Kirschen oder für eine rasche Überprüfung unsicherer Ergebnisse, die bei einem Test mit Pfirsichsämlingen erzielt wurden. Wenn

auch mit dieser Schnellmethode keine hundertprozentige Sicherheit des Virusnachweises erreicht werden konnte und wohl auch gegenwärtig nicht zu erreichen ist, so stellt sie meines Erachtens doch eine wertvolle Ergänzung der gebräuchlichen Nachweisverfahren dar, in denen Gehölze als Indikatoren verwendet werden.

ZUSAMMENFASSUNG

Gurken, Tabak und *Chenopodium*-Arten zeigen schon 4-14 Tage nach der Infektion mit einigen Steinobstviren Symptome. Es wurde deshalb geprüft, ob diese Pflanzen als Testpflanzen für einen Virusnachweis beim Steinobst geeignet sind. Eine mechanische Übertragung der Viren von Kirschen oder Pfirsichen auf Gurken gelingt verhältnismäßig leicht, wenn als Infektionsmaterial aufbrechende Knospen von Kirschen oder junge, gefaltete Pfirsichblätter verwendet werden. Allerdings muß diesen Pflanzenteilen vor dem Zerreiben etwas Wasser oder Phosphat-Puffer zugesetzt werden.

Gurke ist ein guter Indikator für unsere einheimischen Kirchenviroseu mit Ausnahme der Pfeffinger-Krankheit. In Versuchen konnte an 38 von 43 infizierten Kirschen- und Pfirsich-Sämlingen Virusbefall nachgewiesen werden, wenn für jeden Test 10 Gurkenpflanzen verwendet wurden und die Temperaturen des Gewächshauses 25°C nicht überschritten. Mit Gurken als Testpflanzen können auch Virusstämme der Kirschen-Ringflecken-Krankheit erfaßt werden, die auf Pfirsich als Testpflanze keine oder nur undeutliche Symptome hervorrufen. Tabak entwickelt nach der Infektion mit dem Virus der Pfeffinger-Krankheit Symptome, die dem Krankheitsbild der Tabak-Ringflecken-Krankheit entsprechen. Ähnliche Symptome treten am Tabak auch nach der Infektion mit einigen latenten Kirschenviren auf. Da aber die Symptomausbildung des Tabaks ab Anfang April unsicher ist, eignet er sich wenig als Testpflanze für Kirschenviren.

Ein sehr guter Indikator für die Pfeffinger-Krankheit ist *Chenopodium quinoa*. Die Pflanze entwickelt lokale und schwere systemische Symptome und reagiert empfindlicher als Tabak oder *Chenopodium amaranticolor* auf diese Virose. Das Virus der Stecklenberger-Krankheit erzeugt ebenfalls Symptome auf *Chenopodium quinoa*.

SUMMARY

Cucumbers, tobacco and *Chenopodium* species develop symptoms after a period of 4-14 days following the infection with some stone fruit viruses. Therefore it was investigated, if these plants are good indicators for indexing stone fruit trees for the presence of virus. The mechanical transmission of viruses from cherries or peaches to cucumbers is quite successful, if raising buds of cherries or young folded leaves of peaches are used. Before grinding, however, water or phosphate-buffer must be added to the plant tissue.

Cucumber is a good indicator for the endemic virus diseases of cherries with the exception of the "Pfeffinger" disease. In experiments 38 out of 43 virus-infected mazzards and peach seedlings gave positive reaction, if 10 cucumber plants were used for each test and if the temperature of the green-house did not exceed 25°C. With cucumbers as indicators we are able to determine virus strains of the cherry ring spot disease, which produce none or only doubtful symptoms on peach seedlings.

Tobacco develops symptoms of the necrotic ring spot type after an infection with the virus of the "Pfeffinger-disease". Similar symptoms may appear after the infection with some latent viruses of cherries. But the expression of these symptoms on tobacco is very uncertain after the beginning of April. Therefore this plant is less qualified as test plant for cherry viruses.

A very good indicator for the "Pfeffinger-disease" is *Chenopodium quinoa*. This plant shows local lesions and strong systemic symptoms and is more sensitive than tobacco plants or *Chenopodium amaranticolor* to this virus disease. The virus of the "Stecklenberger-disease" also produced light systemic symptoms on *Chenopodium quinoa*.

DISCUSSION

Pfaeltzer: We carried out some bud inoculations from Pfeffinger diseased cherries as well as from cherries with ringspot virus. The Shirofugen reacted to both of them. The experiments comprised too few trees however to be able to conclude anything about the rates of the reactions in tests.

Kegler: We also investigated this year the transmissibility of Stecklenberger virus of sour cherry and a ringspot virus of sweet cherry from about 30 sour cherry and sweet cherry trees into cucumber, and we found that the maximum reliability was about 60 per cent. This kind of "cucumber test", I think, is not suitable for indexing cherry mother trees.

Nyland: Has anyone tested the efficiency of cucumber versus Shirofugen for ringspot test? Shirofugen is more sensitive than cucumber for ringspot indexing in U.S. tests.

KUNZE: I have not made tests on Shirofugen till now. Therefore I am not able to decide if cucumber is more or less sensitive for cherry ringspot virus and latent viruses of cherries than Shirofugen.

SCHMID: Different strains of Pfeffinger disease gave negative reaction when indexed to shirofugen while positive reaction was obtained by juice transmission to cucumber.

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Untersuchungen über die Beständigkeit einiger Obstviren in vitro

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Die mechanische Übertragung von Kirschenviren gelingt nicht immer mit genügender Sicherheit. Der Infektionserfolg unterliegt, in Abhängigkeit von verschiedenen Faktoren, großen Schwankungen. Diese sind in der Regel umso größer, je geringer die Beständigkeit des Virus in vitro ist.

Einige Faktoren, welche die Infektiosität alternder Preßsäfte beeinflussen, wurden von Willison und Weintraub (1953) untersucht. Sie fanden, daß Herkunft, Temperatur und Verdünnung des Inokulums, Alter und Wachstumsbedingungen der Wirtspflanze sowie die Jahreszeit die Beständigkeit von Kirschenviren (cherry yellows, chlorotic ring pattern, green ring mottle, necrotic ring spot, prune dwarf, tatter leaf) in vitro verändern können. Baumann (1959) stellte beim Stecklenberger- und beim Ringfleckenvirus fest, daß die Beständigkeit beider Viren bei 2° C größer war als bei 18° C. Hampton und Fulton (1959) fanden Substanzen, welche die Stabilität von Kirschenviren in Gurkenpreßsäften erhöhten.

In der vorliegenden Arbeit wurde der Einfluß von Jahreszeit, Temperatur und Chemikalien auf die Beständigkeit des Stecklenberger- und des Pfeffinger Virus untersucht.

Material und Methoden

In allen Versuchen wurden die gleichen Virusisolierungen verwandt (Stecklenberger Virus (StV): Schattenmorelle 373A; Pfeffinger Virus (PfV): *Prunus avium*-Sämlinge 989 z, 990 z). Von

zwei Bäumen bzw. von mit gleichen Isolierungen infizierten Pfirsichen wurden die betreffenden Viren auf Gurke (*Cucumis sativus* L., Sorte »Delikateß«) übertragen und vermehrt. Die Preßsäfte wurden sofort nach ihrer Gewinnung 1:1 mit 0,1 m Sörensen-Phosphatpuffer (pH 6,9) bzw. mit 0,1 Prozentigen Lösungen (pH 6,8) verschiedener Chemikalien verdünnt. Zur Prüfung der Infektiosität von *Prunus*-Säften wurden junge Blättchen in Reibschalen unter Zusats von Puffer oder Chemikalien zu dünnflüssigen Breien zerrieben.

In jeder Versuchsreihe wurden alle Temperaturstufen bzw. die einzelnen Chemikalien gleichzeitig mit einer kurz zuvor aus etwa 200 Gurkenkotyledonen gewonnenen und unterteilten Preßsaftmenge angesetzt. Die Preßsaft-gemische wurden in Reagenzgläser gefüllt und in unterschiedlich temperierten Wasserbädern aufbewahrt. Diese befanden sich bei 2 und 12° C in Kühlzellen, bei 22, 32, 42 und 52° C in Höppler-Ultrathermostaten. Von jeder Probe wurden in bestimmten Zeiträumen mechanische Übertragungen ohne Karborund auf jeweils 9 Gurken vorgenommen. Die Versuche wurden zwei- bis dreimal wiederholt.

Ergebnisse

Die von August 1959 bis Juni 1960 durchgeführten Versuche zeigten, daß die Beständigkeit der beiden Kirschenviren im Gurkenpreßsaft durch den Einfluß verschiedener Faktoren beträchtlich verändert werden kann.

1. EINFLUSS DER JAHRESZEIT

Bei gleicher Versuchsanordnung war festzustellen daß StV-haltige Preßsäfte in den Herbstmonaten ihre Infektiosität schneller verloren als im zeitigen Frühjahr. In den Monaten Oktober bis Dezember blieb das StV bei 22° C und Pufferzusatz höchstens 6 Stunden aktiv, während es sich in den Monaten Februar bis Mai noch nach 20 h übertragen ließ. Während der Sommermonate konnten keine diesbezüglichen Versuche durchgeführt werden, weil durch zeitweilig hohe Gewächshaustemperaturen der Infektionserfolg unabhängig von der Alterung der Preßsäfte stark herabgesetzt war.

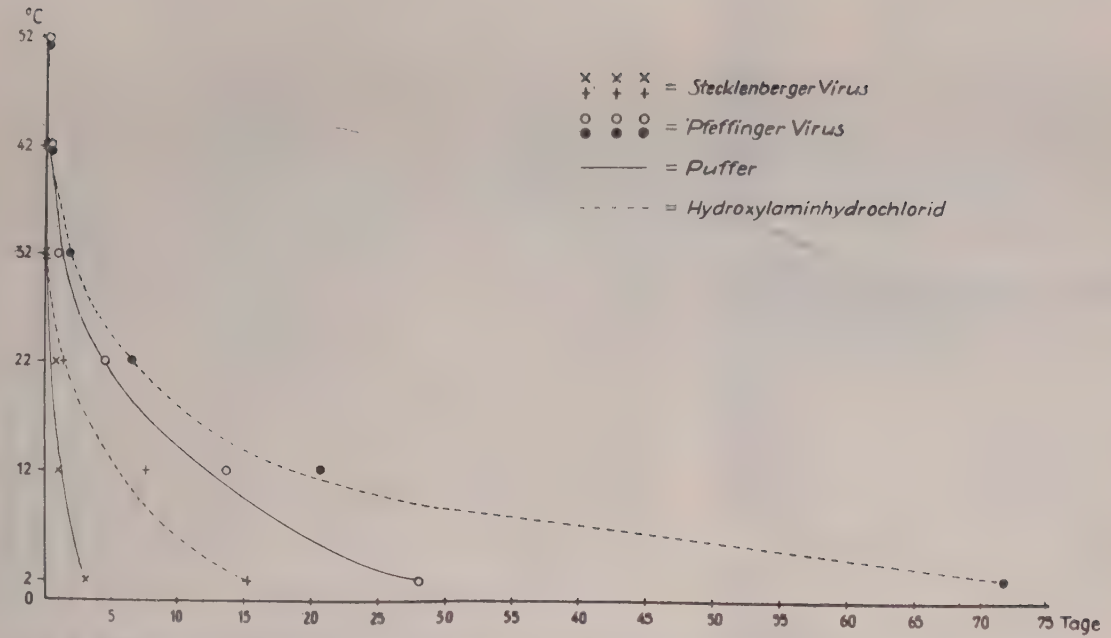

Abb. 1. Beständigkeit des Stecklenberger- und Pfeffinger Virus in vitro in Abhängigkeit von Temperatur und Chemikalien

2. EINFLUSS DER TEMPERATUR

Die Beständigkeit der Viren in vitro steht in engem Zusammenhang mit der Temperatur des Preßsaftes. Obwohl die Wärmeempfindlichkeit von StV und PfV unterschiedlich ist, zeigten beide Viren bei steigenden Temperaturen abnehmende Stabilität (Abb. 1). Das StV ist bei 2° C bis zu 3 Tagen, bei 52° C nur 10 Minuten aktiv. Das PfV verliert seine Aktivität bei gleichen Temperaturen nach 28 Tagen bzw. nach 5 Stunden. Die Beständigkeit im Preßsaft nimmt mit steigenden Temperaturen nicht gleichförmig ab. Der aus den Grenzwerten der Beständigkeit hervorgehende Kurvenverlauf gleicht einer Exponentialkurve.

Die Aufbewahrung der Preßsäfte bei ÷ 8°C führte beim StV bereits nach 4 Stunden, beim PfV nach 48 Stunden zur Inaktivierung.

3. EINFLUSS VON CHEMIKALIEN

Auf Grund der Annahme, daß Oxydationsprozesse zur Inaktivierung der Viren im Preßsaft beitragen (Hampton und Fulton 1959), wurde der Einfluß einer Reihe von Substanzen untersucht, die Oxydationsvorgänge hemmen (James 1953). Die Chemikalien wurden hinsichtlich ihrer Wirksamkeit bei zwei Temperaturstufen untersucht, da die starke Zunahme der Beständigkeit der Viren bei niederen Temperaturen Abweichungen im Wirkungsgrad der Substanzen vermuten ließ.

Zwischen dem zum Vergleich dienenden Puffer und den verschiedenen Chemikalien traten bei 22° C nur geringfügige Unterschiede auf. Deutliche Unterschiede waren dagegen bei 2° C zu erkennen (Abb. 2). Hier verlängerte Hydroxylaminhydrochlorid (H) im Vergleich zum Puffer die Beständigkeit beider Viren erheblich. Während das StV unter Pufferzusatz nur 3 Tage aktiv blieb, lag die Grenze der Beständigkeit bei Zusatz von H bei 15 Tagen. Das unter Pufferzusatz bei gleicher Temperatur 28 Tage infektiöse PfV war unter Zusatz von H noch nach 72 Tagen aktiv (Abb. 3). Semicarbazidhydrochlorid erwies sich nur beim StV bei 2° C als stabilisierend, in dem es seine Beständigkeit von 3 auf 7 Tage verlängerte.

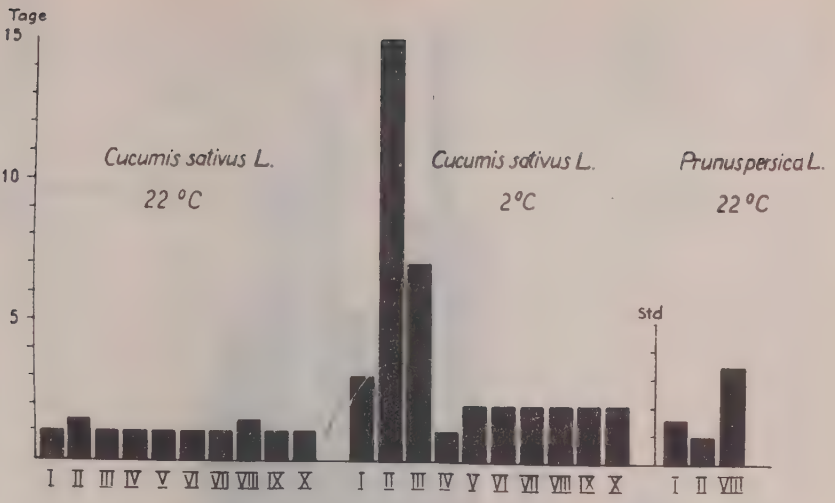

Abb. 2 Beständigkeit des Stecklenberger Virus in vitro in Abhängigkeit von Wirtspflanzen, Chemikalien und Temperatur. Zeichenerklärung:

I = Puffer	VI = Resorcin
II = Hydroxylaminhydrochlorid	VII = ß-Naphtalinsulfosäure
III = Semicarbazidhydrochlorid	VIII = Natriumdiäthyldithiocarbamat
IV = Natriumfluorid	IX = Jodessigsäure
V = Natriumazid	X = 8-Hydroxychinolin

Auf Grund des günstigen Einflusses von H auf die Beständigkeit beider Viren wurde diese Substanz in die Versuche über den Einfluß der Temperatur einbezogen. Hierbei bestätigte sich die bevorzugte Wirksamkeit der H-Lösungen bei niederen Temperaturen. Die Unterschiede zwischen Preßsaft- Puffer- und Preßsaft-H-Gemischen gingen bei höheren Temperaturstufen zurück und fehlten beim StV oberhalb 32° C, beim PfV oberhalb 42° C völlig (Abb. 1).

4. EINFLUSS DER WIRTSPFLANZE

Bereits Willison und Weintraub (1953) stellten fest, daß das cherry yellows-Virus im Gurkenpreßsaft länger aktiv blieb als im Pfirsichpreßsaft. Ein ähnliches Ergebnis war bei den von uns

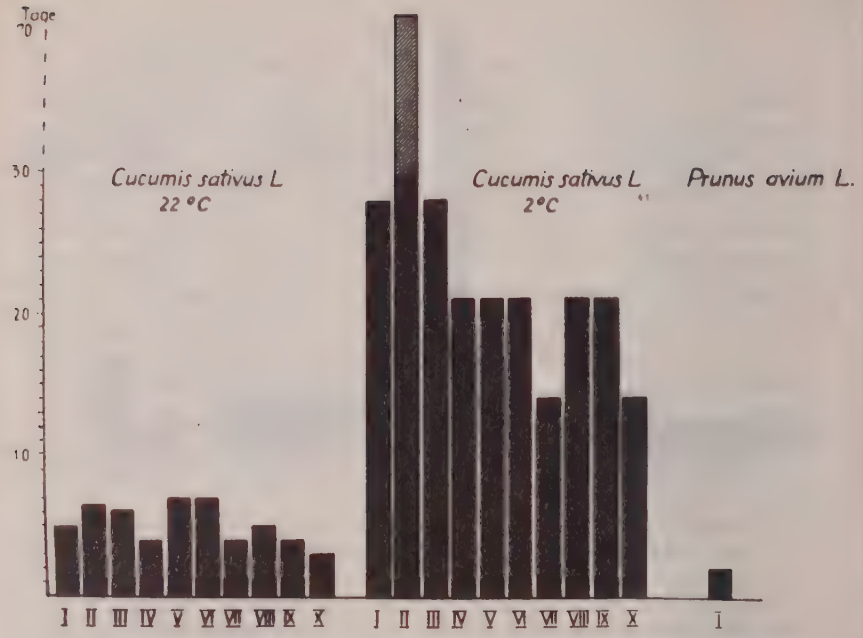

Abb. 3. Beständigkeit des Pfeffinger Virus in vitro in Abhängigkeit von Wirtspflanzen, Chemikalien und Temperatur (Zeichenerklärung siehe Abb. 2)

untersuchten Viren zu erwarten. Ungewiß blieb jedoch, ob H auch in *Prunus*-Säften stabilisierend wirkt.

Wie aus Abb. 2 ersichtlich, lag bei Pfirsichpreßsäften die Beständigkeit des StV bei H-Zusatz niedriger als bei Pufferzusatz. Demgegenüber erwies sich Natriumdiäthyldithiocarbamat (»Dieca«, JAMES 1953) als stabilisierend. Es erhöhte die Beständigkeit des StV von 1-2 (Pufferzusatz) auf 3-4 Stunden. Auch der Infektionserfolg war bei Abreibungen des StV von *Prunus avium* L. oder *P. persica* L. auf Gurke bei Verwendung von »Dieca« höher als bei Puffer- oder H-Zusatz (Abb. 4). Demgegenüber ließen sich bei der Verwendung von Gurkenpreßsäften hinsichtlich des Infektionserfolges keine eindeutigen Unterschiede bei den verschiedenen Chemikalien feststellen.

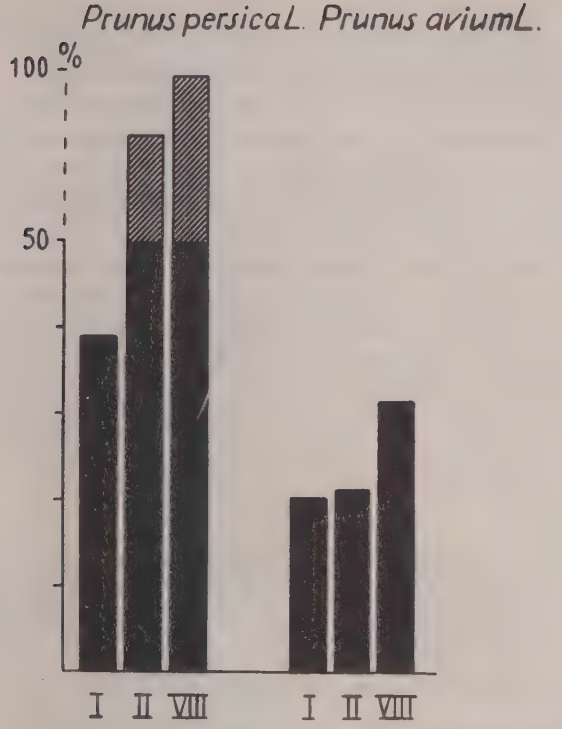

Abb. 4 Infektionserfolg an Gurke in Abhängigkeit von Wirtspflanzen und Chemikalien. (Zeichenerklärung siehe Abb. 2)

DISKUSSION

Die Instabilität von Kirschenviren in Gurkenpreßsäften wird auf oxydierte Polyphenole zurückgeführt, die während der Homogenisierung des Gewebes durch Einwirkung von Polyphenoloxydase entstehen (Hampton und Fulton 1959). Der ermittelte Zusammenhang zwischen Beständigkeit der Viren in vitro und Temperatur der Preßsäfte läßt auf temperaturbedingte Änderungen der Fermentaktivität schließen. Bei 52° C ist zusätzlich, bei ÷ 8° C ausschließlich mit Inaktivierung durch Denaturierung des Virusproteins zu rechnen. Die Unwirksamkeit von H bei höheren

Temperaturen ließe sich dadurch erklären, daß die gegebene Konzentration dieser Substanz nicht ausreicht, um die gesteigerte Reaktionsfähigkeit des Fermentes zu kompensieren.

Die Deutung der Unterschiede, die hinsichtlich der stabilisierenden Wirkung von H und »Dieca« in Gurken- bzw. *Prunus*-Säften bestehen, ist schwierig, da bisher nur wenig über die Natur der in diesen Säften vorkommenden Hemmstoffe bekannt ist. Es ist nicht ausgeschlossen, daß bei Verwendung veränderten Konzentrationen dieser Substanzen diese Unterschiede nicht mehr festzustellen sind. Konzentrationsunterschiede könnten auch zur Erklärung dafür herangezogen werden, daß Hampton und Fulton (1959) durch »Dieca« die Stabilisierung des *Prunus*-Virus B im Gurkenpreßsaft gelang, während dieselbe Substanz in eigenen Versuchen nur beim StV bei 22° C die Beständigkeit geringfügig erhöhte.

SUMMARY

Mechanical transmissions of cherry viruses not always succeed with sufficient certainty. The infection rate depends on many factors and large fluctuations occur. Mostly this fluctuations are the larger the less the stability of viruses in vitro is. Some of these factors which influence stability of Stecklenberger virus of sour cherry (StV) and Pfeffinger virus of sweet cherry (PfV) in plant juice have been investigated.

The experiments were carried out with distinct isolations of StV and PfV. Plant juice was obtained by pressing 200 cucumber cotyledons, and immediately after the juice was subdivided in several portions. Each of them was diluted 1:1 by 0.1 m Sørensen-phosphate-buffer (pH 6.9) or by 0.1 per cent solutions of reducing chemicals (pH 6.8). The single portions were stored at temperatures of + 2, 12, 22, 32, 42 and 52°C. With accurate time intervals inoculations were carried out to 9 cucumber plants in each case. The experiments were repeated two to three times.

In the same arrangement of the experiments StV during the spring months was more stable than in autumn. From October to December this virus was active up to 6 h at 22°C juice temperature. From February to May it was still transmissible after 20 hours storage.

Further the stability of both viruses depends on temperature of inoculum. The stability decreases with increasing temperatures. For example StV was active at 2°C up to 3 days but at 52°C only for 10 minutes. PfV was found to be more stable than StV but it was showing the same tendency. At 2°C it was active for 28 days and for 5 h at

52°C. The decrease in activity of both viruses was following an exponential curve.

Since oxydation processes in plant juice probably cause the inactivation of viruses, the influence of some substances which oxydations inhibit was investigated. The efficiency of these substances was investigated at two degrees of temperature (2 and 22°C). At 22°C only small differences between buffer and the inhibitors were established, while great differences occurred at the lower temperature. Especially addition of hydroxylaminehydrochloride (H) caused a stabilisation of both viruses in cucumber sap. While StV in buffer solution kept at 2°C was active in 3 days only, in H solution its activity continued up to 15 days. The same chemical solution prolonged the activity of PfV from 28 up to 72 days. Semicarbazidehydrochloride showed a smaller stabilizing effect, but only with StV. The other substances such as sodium fluoride, sodium azide, resorcinol, β-naphtalene-sulphonic acid, iodoacetic acid, and 8-hydroxyquinoline were completely inactive in 0.1 per cent solutions.

In juice of *Prunus* leaves diethyldithiocarbamate (dieca) showed a stabilizing effect. The infection rate also increased if Prunus leaves had been homogenized with dieca.

DISCUSSION

Blencowe: Did any variability in symptom type occurs, when inocula were treated to near their thermal inactivation point?

Kegler: No, the symptoms were always the same.

Schmid: Did you use material from different sources of the two diseases Stecklenberger- and Pfeffinger-disease?

Kegler: One distinct source in each case has been used because the extent of this investigation was too large to try several sources simultaneously.

Fulton: We find differences in stability of *Prunus* viruses at different times of the year. These appear to be due to differences in composition of the extracts rather than differences in virus content.

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Experiments on the „Line Pattern" of the Cherry in Italy

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Numerous viruses have been encountered in Italy in recent years on fruit trees, including the cherry.

In some zones of the provinces of Rome and Bari, a disease of the sweet cherry of the "line pattern" type is present. It has been observed on plants of the Francia and Ravenna tardiva varieties.

Spots of pale green which then become creamy yellow or whitish-yellow appear on the leaves. The form and size of the spots are variable, but they often consist of well defined, sinuous or irregular lines, or fringed and symmetrical designs on single veins. They can also be small and rounded or ring-shaped. Many leaves on the same plant show such injuries. The summer temperature attenuates or impedes the appearance of the disease on the new vegetation.

In Europe, diseases of the cherry characterized by leaf mosaic or stripes or line and ring spots which show some analogy to that mentioned above, encountered in nature, are attributed mostly to the "ring spot" virus group and have been described, with various denominations, by many authors (for example, Atanasoff, 1935; Mulder, 1955; Baumann and Klinkowski, 1955; Boek, 1956; Kristensen, 1956; Christoff, 1938, 1958; Canova, 1959).

The symptoms we observed recall somewhat those described by Schuch (1957, 1959) for the "linear and ring mosaic" (Ring und Bandmosaik) of the cherry, a disease which seems to be produced by a virus different to that of the "linear and ring mosaic" of the plum and which is considered nearer to the "chlorotic mottle" or "ring mottle" of the cherry recorded by Posnette (1954) and by Posnette and Cropley (1956).

Given the importance of the cultivation of the cherry in Italy, we have proposed to investigate the nature and some characteristics of this disease, and we have sought to learn the relationship between it and other diseases of fruit trees.

The peculiar symptomatology and the evident absence on the attacked plants of other causes to which to attribute the deterioration have facilitated the orientation of the research in the field of the viruses.

The experiments made are the following:

1) *Diagnostic ascertainment*

Grafts made in the summer of 1958 on cherry trees of the *Ravenna precoce* variety and on wild cherry trees coming from seed have determined, both in the first instance and, to a great extent, in the second, the appearance of pathological symptoms analogous to the original ones. The disease not only reproduced itself on the vegetation obtained from the grafts but was transmitted to the grafted plants and appeared on many leaves. The virus etiology of the disease has thus been demonstrated experimentally.

2) *Transmission to other species of fruit trees*

a) *Apricot.* The disease has been transmitted, by budding and chip-budding, to apricot trees of the Luizet variety, producing symptoms similar to those of the "line pattern" of the apricot, a disease also present in Italy (Gualaccini, 1959). This virus has proved transmissible also to the cherry; the appearance of the pathological symptoms took place in the second year after grafting. Probably the two disases have been produced by strains of the same virus.

b) *Almond.* The transmission of the virus has been obtained, by chip-budding, on almond trees of the Della Regina variety. The symptoms created on them are referrable to the disease known in Italy as almond "mosaic", of which various forms and strains of the virus have been individuated by Scaramuzzi (1956, 1957). On the other hand, we recall that the "mosaic" of the almond has also been transmitted to the cherry from seed and to the Mazzard cherry F 12/1, with production of a slight chlorotic mottling,

often united to deformations and tattering of the leaves. It may be considered, therefore, that the "mosaic" of the almond also belongs to the same virus group of the "line pattern" of the cherry and apricot.

c) *Peach and plum.* Inoculation trials of the "line pattern" of the cherry, made on Shiro and Myrobalan plum trees and on Fior di Maggio and Amsden peach trees, have not had any results because the union of the tissues between the grafted parts did not occur. However, on the basis of the symptomatological analogies indicated above, we observe that both the "line pattern" of the apricot (Gualaccini, loc. cit.) and the "mosaic" of the almond (Scaramuzzi, 1956) proved transmissible also to the peach and plum, producing symptoms of the "line pattern" type.[1]

3) *Tests on indicator plants*

Buds or chips of cherry attacked by virus, grafted or transplanted on trees of *Prunus serrulata var.* Shirofugen and Kwanzan, have not produced any reaction. Similar grafts of the above-mentioned varieties of healthy flowering cherry on cherry attacked by "line-pattern" have vegetated normally, in the first year of grafting. This leads to the consideration that the virus of "ring spot" is extraneous to the disease in question and induces the ascription of

1. Without going into the subject in detail here, we may add that there have been encountered similarities in the symptoms produced, on these same fruit-bearing species, not only by the "line pattern" of the cherry and of the apricot and by the "mosaic" of the almond, but also by the "line pattern" of the plum and of the peach, as well as by the "mosaic" of the apple (ex. Christoff, 1938; Posnette, 1953; Kirkpatrick, 1955; Gilmer, 1956; Posnette and Ellenberger, 1957; Corte and Scaramuzzi, 1959), and, in addition, by the "line pattern" of the flowering cherry (Scaramuzzi and Corte, 1957) and by the "yellow mosaic" of the rose. This last, observed in Italy since 1957 (Gualaccini, 1958) has been recently transmitted by us to the myrobalan plum and to the apricot. For the bibliography on the "line pattern" of the plum and of the peach, we limit ourselves to recording Christoff (1958) and Baumann (1959) and we recommend particularly references to the papers of Scaramuzzi and Corte (loc. cit.) and Posnette and Ellenberger (loc. cit.).

all the symptoms observed on the attacked plants, including the ring-spot types, to the syndrome of "line pattern".[1]

Other varieties of the sweet cherry [Bing, Lambert, Mazzard F 12/1 (E.M. clone)] and of the sour cherry (Montmorency) indicators of the principal viruses of the cherry, grafted on infected cherries and vice-versa, have not shown, over a year after the grafting, the symptoms of the disease, or have shown it in slight measure, except for Mazzard F 12/1, which has shown it abundantly.[2] This last has proved an excellent indicator of the virus.

4) *Tests of some cherry trees of normal appearance situated near other diseased ones*

A cherry of the Ravenna tardiva variety, apparently healthy, situated in the midst of other diseased ones, was tested on the same above-named indicator varieties. The method of double-budding was adopted, i.e. on the stem of a single small wild cherry tree, coming from seed, were budded, contemporaneously, a bud of the Ravenna tardiva (below) and one of the indicator variety (above). The vegetation produced by the buds of the indicator Mazzard F 12/1, and the same vegetation of the wild cherry trees budded with it, have demonstrated symptoms of "line pattern". Shortly after, slight symptoms of the virus were noted also on the tree tested.

Similar tests have also been made on a cherry of the Cerasa Alloro variety, situated not very far from the infected cherry trees, but with negative result.

5) *Trials of transmission to herbaceous plants*

Mechanical inoculation trials have been made on various herbaceous plants: petunia, cucumber, thorn-apple, eggplant, tomato, basil, cowpea, Herzegovina tobacco, *Nicotiana glutinosa*. Rubbing

1. In the case of the "Ring- und Bandmosaik" of the cherry, Schuch (1959) has obtained a weak reaction on Shirofugen (German clone), but it seems that this was produced by the virus of the "ring spot" present contemporaneously on the diseased cherry trees, as from verbal communication made to us by Schuch himself.
2. Gilmer (1957) has established in plants of sour cherry var. Montmorency the presence of a virus which he thinks is probably that of the "line pattern" (Marmor lineopticum Cation), in the latent state.

their leaf surfaces with cotton soaked in the sap of young leaves of visibly infected cherry trees, properly diluted, with the aid of abrasive powder, has not produced any effect.

6) *Transmissibility through the seed*

Observations have been made of small wild cherry trees of one year with symptoms of "line pattern", located near or not very distant from adult cherry trees attacked by the same disease. Some of these proved to have been produced by the roots of the adult cherry trees, others not. We do not know precisely the origin of these latter, but it is probable, as the farmers owning them also told us, that they came from the seeds of the cherry trees attacked by "line pattern", the possibility of root grafts with infected cherry trees being excluded because of the distance.

7) *Attempts at examination with the electron microscope*

Various preparations of the leaf sap of the infected cherry trees, obtained by the "immersion method", have been examined with the electron microscope. This method, as is known, has been used effectively by Brandes (1957) to render evident viruses of the filiform or rod-shaped type.

This has been applied by us also to leaves of the cherry and the almond inoculated with the virus of "yellow mosaic" of the rose and with that of the "ring spot" of the stone-fruit trees. The results of the observations have been negative.

8) *Thermal inactivation*

Twigs of the infected cherry have been immersed in heated water in a double-boiler for different times and at different temperatures. With them have been made buddings and chip-buddings on wild cherry trees of one year, obtained from seed.

From the observations made about two years after the trials, the treatment of the twigs at 45-46° for three hours proved capable of inactivating the virus completely without sensibly damaging the twigs themselves.

In the control test, i.e. by the budding made from twigs not submitted to any thermal treatment, the virus appeared not completely systemic on the cherry of the Francia variety.

SUMMARY

In certain localities of central-southern Italy is present, on the cherry, a disease characterized by chlorotic leaf markings: mottling of the linear-ring mosaic type, flecking, and irregularly diffused areas.

It has been transmitted by grafting to various varieties of cherry and wild cherry coming from seed.

The results of the transmission trials made on the apricot and almond have led to the conclusion that the disease is produced by a virus or by strains of one belonging to the same virus complex which causes the "line pattern" of the apricot and the "mosaic" of the almond in Italy.

Inoculation trials on the Shirofugen and Kwanzan varieties of the *Prunus serrulata* have demonstrated the absence of the virus of the "ring spot" on the infected cherry trees. From their characteristics and from the type of symptoms, it is considered that the disease is produced by the virus complex of the "line-pattern" type. Also the ring type of spots is to be considered a symptom of this virus.

Apparently healthy and growing cherry trees near other infected ones have been tested by means of varieties which are "indicators" of the viruses of the cherry. It has been ascertained in this way that the agent of the viruses can be present even on plants which do not yet show the symptoms.

The Mazzard F 12/1 (E.M. clone) cherry has proved the best indicator of this disease.

The first attempts at mechanical transmission of the virus to different herbaceous plants *(petunia,* cucumber, thorn-apple, ,eggplant pepper, tomato, basil, cowpea, tobacco and *Nicotiana glutinosa)* have given negative results.

The presence of one year old wild cherry trees, showing the symptoms of the virus and growing near infected cherry trees, has led to consideration of the possibility of the transmission of the virus through the seed.

Attempts at examination with the electron microscope of preparations obtained with the "immersion method" have been negative.

Tests of thermo-therapy with immersion in heated water, on twigs of infected plants, have shown that the virus can be completely inactivated by treatment at 45-46° C. for three hours.

DISCUSSION

NYLAND: Gualaccini's line pattern of cherry looks like our almond calico on cherry, but our culture does not show strong symptoms beyond the first year after inoculation. Our almond calico on Shiro plum produces only oak-leaf symptoms and mosaic, but no vein banding.

Anonymous: I would like to ask whether either Dr. Gualaccini or Dr. Nyland has infected apple with almond line pattern or almond calico?

Gualaccini: I have not infected apple.

Nyland: Only one source (out of three) of calico virus produced symptoms in apple and this one was contaminated with cherry rugose mosaic.

Almond calico does not cause complete symptoms of line pattern in Shiro plum, vein banding being absent.

Anonymous: Dr. Gualaccini suggested that his cherry disease is similar to the English ring mottle of cherry. The latter virus, however, does not produce symptoms in peach.

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Observations and researches on viruses and viroses of fruit trees in Emilia region

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The Institute of Plant Pathology of Bologna University has been working for several years - ever since it was re-established after the second world war - through studies and researches on virus diseases of fruit trees and on their agents.

This work has been remarkably intensified in recent years according to a program where the practical part of the phytopathological problem is emphasized, because of the ever increasing quantity of virus diseases found in fruit trees and especially in the propagating and commercial material; we think in fact, that the trade in nursery material among the regions of our country and with foreign countries, made in the past without any control on the viroses, is responsible for the unhealthy conditions of our fruit-tree plantations.

At the present moment, because of several observations made in many orchards and nurseries in Emilia, we have a fairly exact view of the situation which enables us to work better in consequence.

In short, the main points of our activity and the results obtained till now may be summed up as follows:

Spread of the diseases

It has been proved that the pear mosaic, especially in some varieties such as "Coscia" and "Williams", is by far the most common virosis. Out of this disease we distinguished some symptomatological forms ("veinal mosaic", "chlorotic spot", "ring spot", "red spot", and "necrosis and deformation") which are of the same kind as in the other countries.

In most cases the mosaic is easily detected by a simple inspection both in young plants and in adult ones; among the various forms of disease the "simple spotting", the "ring mosaic" and the "veinal mosaic" are the most common.

As to the influence of the mosaic on the development of young plants of pear trees, we have observed that the infected plants may be considerably less developed by about 15-35 per cent than the healthy plants.

On the second place, as to the virosis spreading there is the peach necrotic ring-spot, which appears a little in all the drupaceous plants but in particular in sweet cherry. The symptoms of this virus do not always correspond to the typical ones which we normally ascribe to, and frequently they are completely absent, especially in adult plants.

In some varieties of cherry infection by this virus appears essentially as small necrotic spots and perforation of the leaves, in some periods of the years and in some growing stages of the plants.

Several viroses of a different kind have been detected in fruit trees in our country; they are chiefly on apple trees: mosaic, flat-limb, rubbery-wood, proliferation virus and some kinds of alterations of the fruits; these last cannot yet be included in the forms of this kind described so far; on drupaceous plants: mosaic, rosette, chlorotic spots of peach, dwarfing and linepattern of plum and tatter-leaf of the cherry. Also in these plants we have detected anomalies of the fruits caused by viruses, which do not seem identical with those detected and described by other authors.

The health conditions, as regards virus diseases, of the most common root stock, whether obtained from seeds or by vegetative propagation, seem satisfactory enough on the basis of the tests made op till now. But we have observed some cases of infection of pear mosaic on quince, of rubbery-wood on East Malling IX and relatively high percentages of cases of ring-spot on seedlings of peach trees (about 12-15 per cent).

Notwithstanding several studies declaring that the most important factor in the spreading of the viroses of the fruit trees is the employment of the infected scion-wood, we think that other elements in our country are responsible.

Several field observations repeated many times increase our suspicion of natural spread as in the case, e.g., of pear mosaic, which was almost unknown about ten years ago, and of those viroses which damage the fruits of apple-trees and drupaceous plants and which we see spreading more and more.

Experimental researches

Among the several researches we are performing, we want to record here some that we think are worth mentioning even though they are still in an experimental stage.

They are:

1) A quick test of the virus infections using paper chromatography. We have made experience up till now, only on pear tree (mosaic) and peach tree (ring-spot) by ascending or descending chromatography and using, as a solvent, butylic alchohol, acetic acid and water in proportions of 4:1:5.

   We have observed a good correspondence (about 80 per cent of the cases) of the chromatographic test of healthy and sick leaves (the difference is between the extinction points) so that we hope to be able to use this method extensively.

2) Very good results have been obtained with a simple method of testing based on the vegetative multiplication of the plants by their roots (pear, apples, myrobalan, cherry Mazzard F. 12/1). We take from the plants during the dormant period, some pieces of root about 20 cm in length and about 1 cm in width; we keep those for a short time in a humid place, then we graft them towards the upper extremity with a dowel of tissue taken from the plant which is to be tested.

   The graft is protected by a rubber tape and the inferior extremity of the root-scion is covered with paraffin.

   The pieces of root are set in sand in a sunny and sufficiently hot place. After a period – different from plant to plant (but on the whole of about a month), the buds develop from the root and then, from the opposite extremity, the roots.

   The symptoms on the leaves (of course when the graft has been successful) appear generally a month later. By this system it is possible to make a quick test of the plants lasting

about 3-4 months; the place occupied by the scions is very small, which is a remarkable advantage.

The best results have been obtained with root-scions of *Pyrus betulaefolia* (pear mosaic), myrobalan (plum line-pattern) and also of apple (apple mosaic); on *P. betulaefolia* the pear mosaic appears essentially as a interveinal mosaic and red spots.

3) Detection of new indicator plants. The best results, in this research, have been obtained up till now with the *P. betulaefolia* as regard to pear mosaic, towards which this plant appears very sensitive. The inoculated plants react to the infection in a short time (two or three months) and show an interveinal mosaic and an interveinal red spot in conformity with the virulence of the strain of virus used.

Control and test of nursery material

Our activity in this section of resarch on virus diseases of fruit trees is made both by visual inspection and by objective test on indicator plants. In this last case we follow the instructions and employ the test-plants that are fit for the purpose.

We have intended:

1) to identify virus-free strains of plants
2) to reproduce those, under proper conditions, to grow mother-plants, producing grafting material.
3) to test production of nursery material for commercial use by employment of virus-free buds, grafts and root stocks.

Notwithstanding the short period of time from the out-set of this activity, we have succeded in identifying numerous virusfree plants of the most valued varieties cultivated in our orchards especially of pear and apple trees. We have started to make orchards of mother-plants and to produce graftwood that will be sold on the market next year for the first time.

The whole of our research, and in particular the control and testing of the nursery material, is made with the assistance of the Ministero dell'Agricoltura e Foreste and of private fruitgrowers.

Presentation des symptômes de quelques maladies a virus des arbres fruitiers rencontrées en France

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L'étude des maladies à virus des arbres fruitiers en France est récente, puisque c'est seulement au cours de l'année 1956 que nous avons effectué les premières explorations systématiques dans les vergers pour déterminer quelles maladies existaint et quels problèmes elles posaient.

Si l'on veut rapidement indiquer la situation sanitaire de la France telle qu'elle ressort des enquêtes des premières années, on signalera l'existence des maladies classiques suivantes:

Sur *Abricotier, Pêcher* et *Prunier*, mosaïque du type Plum line pattern. En particulier, certains Pruniers porte-greffes multipliés végétativement en sont très nettement et très fréquemment atteints et il semble que ces Pruniers soient à l'origine de la contamination de ces 3 espèces.

Sur le *Cerisier*, fréquemment, les taches annulaires chlorotiques, les taches annulaires nécrotiques, les énations (Rasp leaf).

Sur le *Pommier*, la mosaïque assez rare, contrairement à celle du Prunier, la *difformité des branches* (Flat limb), rare également, le *bois caoutchouc* (Rubbery wood), signalé dans quelques vergers (sans susciter jusque-là l'inquiétude des arboriculteurs).

Sur le *Poirier*, la *Gravelle* assez fréquente et quelques cas de *mosaïque* ou d'*anneaux jaunes*.

Après avoir cité ces maladies classiques, nous présenterons maintenant quelques maladies moins connues ou particulièrement graves. Ceci nous permettra d'indiquer quelles sont actuellement les préoccupations majeures concernant, en France, les maladies à virus des arbres fruitiers.

I - Arbres fruitiers a noyaux

1.) *Abricotier:*

Le Dépérissement de l'Abricotier a constitué le sujet initial de l'étude des viroses des arbres fruitiers en France. Nous avons fait, au deuxième symposium en 1955, un exposé relatant les observations et quelques expériences préliminaires sur ce sujet. Notre but n'était pas de présenter des résultats certains, mais essentiellement d'attirer l'attention de nos collègues d'autres nations sur la possibilité de présence de virus parmi les facteurs responsables du phénomène complexe du dépérissement de l'Abricotier. Bien qu'une mise au point compiète n'ait pas encore été publiée, tant sur les symptômes que sur les expériences de transmission, nous avons signalé à plusieurs reprises dans des publications partielles l'existence et les principaux caractères de cette virose.

Nous tenons à bien préciser que nous n'attribuons pas la totalité des accidents de dépérissement à cette virose. Cependant, à côté des maladies diverses, et en particulier de la Verticilliose, cette virose est responsable, au moins dans certaines régions, de

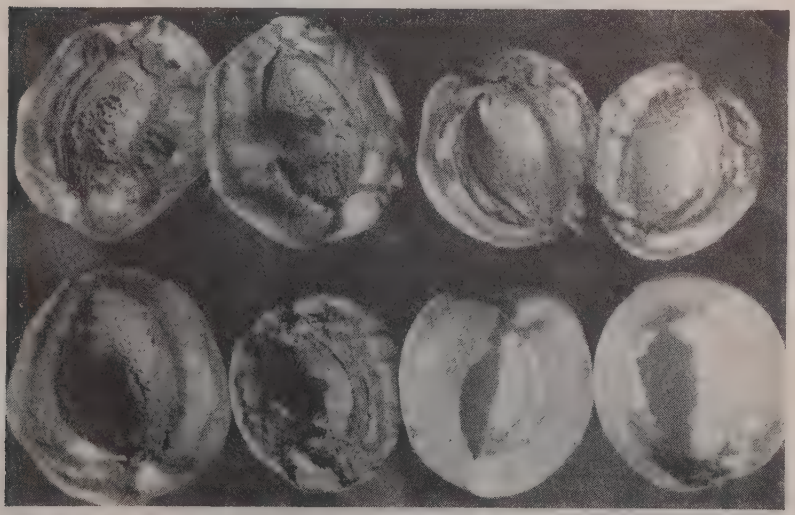

Fig. 1. Dépérissement de l'Abricotier. Brunissement de la chair du fruit. En bas à droite, deux demi-fruits normaux.

la mort de la majorité des arbres. C'est précisément le cas dans des vergers greffés sur Prunier, sur une terre qui n'a pas porté de plantes herbacées hôtes du *Verticillium dahliae*.

Voici ces principaux caractères.

Symptômes d'été:

Symptômes sur Fruits: épiderme ridé brunissement de la chair à partir du noyau, allant jusqu'au dessèchement du fruit sur l'arbre Visible presque uniquement dans la région lyonnaise, rare dans le Roussillon. (Fig. 1).

Symptômes sur le feuillage: enroulement des feuilles jusqu'à former un cornet ou enroulement suivant 3 à 5 lignes avec jaunissement irrégulier entre les nervures.

Développement irrégulier de la végétation en été avec reprise en général fin août.

Symptômes d'hiver:

Fig. 2. Départ précoce de la végétation. Le rameau gauche provient d'un arbre inoculé avec la maladie, celui de droite d'un arbre témoin.

Le depart de la végétation en hiver peut permettre un développement complet du feuillage au moment normal du débourrement. (Fig. 2).

Fig. 3. Nécrose du liber sur un arbre inoculé.

Ce départ précoce provoque le gel partiel ou total des branches, entraînant une nécrose du liber plus ou moins profonde (Fig. 3) et des crevasses longitudinales de l'écorce tuée par le froid sous la poussée des tissus formés à partir du cambium demeuré vivant.

Il nous reste en particulier:

à distinguer différentes formes de la maladie; à préciser les réactions du pêcher à la maladie (résistance par hypersensibilité ou apoplexie brusque un an après l'inoculation); à trouver un indicateur valable; à étudier comment la maladie se perpétue.

2.) *Pêcher:*

Nous avons relevé et transmis des symptômes à rattacher au type *rosette*. L'une de ces formes particulièrement grave affecte les

pêchers des Monts du Lyonnais. Nous l'avons dénommée *court-noué* pour ne pas préjuger d'une identité avec la maladie désignée sous le nom de Rosette aux Etats-Unis. (Fig. 4).

Fig. 4. Court-noué du Pêcher.

Les bourgeons sont rapprochés, les feuilles légèrement enroulées et tordues, le porte-greffe, en rejettant, peut former un petit buisson, la floraison est retardée, les fruits à maturité tardive sont plus gros et peu nombreux.

D'après les dernières observations, cette maladie paraît provoquée par une virose qui se serait d'abord attaquée aux vieux cerisiers qui ont été arrachés ou sont demeurés sur le terrain où ont été plantés les pêchers.

La chlorose du Pêcher se rencontre en différentes zones de culture du Pêcher, amenant les arbres à la stérilité, sinon à la destruction. Frappé par le contraste des arbres demeurés bien verts au voisinage immédiat des arbres malades, et nous inspirant des indications données par notre collègue Bovey, nous avons essayé à plusieurs reprises des inoculations de Pêcher avec des écussons ou des tissus de Pêchers chlorosés.

Dans un certain nombre de cas, mais sans manifester de chlorose, les arbres inoculés ont présenté un développement nettement plus faible et surtout des porte-greffes S 305-1 inoculés avec des Pêchers chlorosés provenant des Alpes-Maritimes ont manifesté dès l'année suivante un très net enroulement du feuillage, particulièrement visible un mois après le départ de la végétation.

Il reste à préciser l'identité des virus mis en évidence et dans quelle mesure ils concourent à l'apparition de la chlorose.

3.) *Cerisier:*

Les symptômes des énations se rencontrent sur des arbres qui paraissent être atteints de maladies différentes.

Tantôt ce sont de petits massifs de tissus un peu verruqueux sans forme définie, à l'aisselle des nervures principales et secondaires, tantôt ce sont des poils raides sur toute la surface inférieure, tantôt des excroissances ayant la structure des bords de limbe accompagnée de déformation considérable de la feuille.

L'effet sur la croissance est extrêment variable.

II. Arbres fruitiers à pépins

1. *Poirier:*

Nous avons mis en évidence une virose qui ne nous paraît pas avoir été signalée.

Cette maladie est apparue dans les circonstances suivantes:

Des poiriers de la variété Doyenné du Comice, greffés sur Cognassiers, avaient été surgreffés avec simultanément les variétés Beurré Clairgeau et Beurré Giffart. Alors que la plupart des arbres issus de l'association étaient normaux, certains présentaient un développement anormal de la variété Doyenné du Comice devenue intermédiaire. Au printemps, presque aucun bourgeon ne se développait et la plupart se détachaient. Un ou 2 mois plus tard seulement, des bourgeons perçaient à proximité de cicatrices et donnaient naissance à des pousses assez chétives.

En vue de préciser la cause de ces anomalies, des portegreffes poiriers de semis furent greffés avec des écussons de la variété Doyenné du Comice provenant d'arbres d'aspect normal; un

écusson provenant d'un arbre anormal, soit de la partie Doyenné du Comice, soit de la variété Beurré Clairgeau ou Beurré Giffart, était placé à proximité sur le même porte-greffe. Les témoins étaient constitués par des porte-greffes greffés uniquement avec Doyenné du Comice issu d'un arbre normal.

On a pu relever par la suite le comportement suivant :

Les greffons de Doyenné du Comice normal ont pratiquement tous donné une pousse normale la première année. Il en est de même des greffons de Beurré Clairgeau.

Sauf rare exception, les greffons de Doyenné du Comice provenant d'un arbre anormal ne se développèrent pas, qui qu'il y ait une soudure excellente et que plusieurs années après, le fragment

Fig. 5. Virose du Poirier. On remarque vers la gauche un rameau de la variété Doyenné du Comice issu d'un greffon placé en haut du porte-greffe. La plupart des bourgeons sont tombés, à peine quelques feuilles se développent. Le porte-greffe et le rameau issu du bourgeon de Beurré Clairgeau greffé plus bas ont une croissance normale.

Fig. 6. Détail d'un rameau de Doyenné du Comice. Le bourgeon normal est détruit, tandis qu'un petit bourgeon se développe latéralement avec retard.

soit encore parfaitement vivant et ait légèrement augmenté de volume.

La seconde année, les témoins (Doyenné du Comice) continuent à croître, ainsi que les pousses de Beurré Clairgeau. Par contre, on constate sur les pousses Doyenné du Comice placées sur un porte-greffe inoculé (portant également un greffon de l'une ou l'autre variété issu d'un arbre anormal) que les bourgeons ne débourrent pas à l'époque normale. Au contraire, la plupart se détachent, laissant une cicatrice très nette. Ainsi, des pousses formées l'année précédente, longues de 60 cm à 1 m portent tout au plus quelques rosettes de 4 ou 5 petites feuilles. Quoique pouvant demeurer sans végétation sur quelques décimètres, le rameau peut rester bien vivant sans trace de nécrose. Parfois ultérieurement, l'extrémité se dessèche lentement (Figs. 5 et 6).

Dans certains cas, quelques bourgeons donnent des pousses grêles de quelques 10 cm, mais l'année suivante, le même phénomène recommence. Les quelques pousses grêles formées l'année précédente généralement ne végètent plus et c'est un ou 2 bourgeons provenant de rameaux plus vieux, qui assurent la pérennité du Greffon Doyenné du Comice. On a pu regreffer en Doyenné du Comice des porte-greffes qui avaient été ainsi infectés. Plusieurs fois, une pousse s'est développée pendant 1 an, puis a cessé de croître l'année suivante, laissant une tige nue.

L'importance de cette maladie paraît difficile à indiquer. A première vue, tout au plus pourrait-elle expliquer certains échecs de greffage. En tout cas, cela vient montrer une fois de plus l'existence de virus latents.

2.) *Pommier:*

La prolifération ou balai de sorcière est une maladie qui a été suffisamment décrite pour que nous n'insistions pas sur ses symptômes. Citons: l'élargissement des stipules, le départ de pousses anticipées, le départ plus précoce des bourgeons avec un certain retard de l'ouverture des fleurs, la diminution de la taille

Fig. 7. Prolifération du Pommier. Développement de la base de l'inflorescence sur les arbres malades. A gauche, deux inflorescences provenant d'un arbre sain.

des fruits et la modification de leur couleur. Nous avons relevé également un allongement de la base de l'inflorescence. Celle-ci peut atteindre 3 cm et porter de petites feuilles nettement développées, au lieu d'écailles. (Fig. 7).

Nous voudrions surtout, à la fin de cette communication, signaler le danger particulièrement grand que cette maladie constitue pour la France.

Si, dans certains vergers, on remarque seulement quelques rares arbres et si la maladie reste stationnaire, on a pu suivre, dans de nombreux autres vergers, une extension de la maladie, après 8 ou 10 années de plantation, particulièrement inquiétante.

Un verger du Massif-Central planté avec 950 pommiers de la variété Reinette blanche du Canada, a montré au cours de 4 années successives la progression suivante: 2 arbres, 58 arbres, 153 arbres, 207 arbres.

La maladie s'est d'abord manifestée dans le Sud-Est et dans le Massif-Central. Ce sont dans ces régions que l'on rencontre les vergers les plus contaminés. Depuis 2 ans, de nouveaux cas ont été signalés à peu près partout, mais il ne s'agit en général que de quelques arbres isolés.

Là encore, se pose le problème de l'origine de la contamination. Lorsque la maladie apparaît pour la première fois dans un verger après 8 à 10 ans de plantation, peut-on estimer que les arbres atteints étaient malades dès le début, mais que la maladie n'est devenue apparente qu'après un certain délai, ou y a-t-il eu contamination par des vecteurs?

SUMMARY

After short information about the occurrence of the more classical virus diseases of stone and pome fruit trees in France, the author considers some diseases which are more economically important or less known.

Apricot trees are very frequently struck by die-back. Among other diseases such as *Verticilliosis*, a virus disease causes the destruction of most trees.

The main symptoms were shown with the aid of diapositives:

Browning of the fruit flesh around the stone.

Leaf roll with irregular interveinal yellowing.

Irregular growth with a new start at the end of August.

Growth starting in winter. At time of normal bud break leaves may be full expanded.

More or less deep destruction by frost of the bark of these too early growing trees.

On *Peach trees* a severe disease noticed since six years in the South-East was called "court-noué". Short internodes, somewhat rolled and twisted leaves, late blooming and maturity are the main symptoms. The disease seems to have occurred firstly among old cherry trees and passed from them to peach trees planted in the same ground.

On *Pear trees* an apparently new virus disease was found to induce bud drop in early spring. Beurré Giffard and Beurré Clairgeau are tolerant. So far the disease was only seen on Doyenné du Comice.

Lastly some information are given on Proliferation disease of *Apple trees:* remarks on symptoms, occurence and spreading of the disease in the orchards.

DISCUSSION

Cadman: What is the pattern of spread of apricot die-back disease in the field?

Morvan: In the Lyon area apricot-trees (on myrobalan rootstocks) apparently remain healthy the first 5-6 years after the planting and then symptoms appear on a few trees.

In the following years the disease spread to many other trees, first of all those in the neighbourhood of the first infected ones.

Luckwill: Do you observe bud-dropping and rosetting of Comice pear, when you work the trees on seedling pear stocks, or are the symptoms manifested only when the trees are grafted on quince?

Morvan: In the fruit farm where this abnormality has been observed the pear trees were worked on quince, but in our trials we have used pear seedlings as rootstocks and also here we obtained symptoms on Comice.

Baumann: Are you quite sure that in several cases of apricot die-back disease are not involved fungi or bacteria living in the vessels and which may be transmitted by grafting too?

Morvan: In some cases we have found, that wilting of apricots were caused by fungi for instance *Verticillium dahliae,* and we have tried to transmit this fungus by grafting but with negative result. The symptoms caused by apricot die-back virus is quite different from those caused by *Verticillium.*

Bovey: In Switzerland there is also evidence, that apricot die-back in many cases is caused by one or several viruses. The disease spreads in patches with the pattern of a soil-borne virus disease.

In experimental transmissions, peach chlorotic leafroll symptoms were induced on healthy peach seedlings infected by chip-bud in-

oculation from diseased apricots. Peach chlorotic leafroll virus, transmitted from naturally infected peach to healthy apricot seedlings, produced typical apricot die-back symptoms. It seems therefore that one type of apricot die-back and peach chlorotic leafroll, are caused by the same virus. Field observations confirm this hypothesis; both diseases have the same rate and pattern of spread, and when the infection spreads in mixed plantings, where peach and apricot grow side by side, both species are affected.

Hidaka: Concerning the spread of rosette disease I suppose an insect vector to be involved – probably an aphid.

Fruit tree virus disease investigations in Uttar Pradesh

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A large number of orchards of peaches, plums, apricots, apples and pears are situated in Kumaon hills of Uttar Pradesh. They are found from an elevation of 2000 ft. (600 meters) to 8000 ft. (2400 meters) above sea level. With such variations in altitude, the range of climatic conditions is extreme. In most of the above area the climate on the whole is temperate. The temperature rises up to 90°F (32,2°C) in summer while in the coldest part of the winter, temperatures as low as 26°F have been recorded.

The average rainfall lies between 150 cms. to 275 cms. in different parts of Kumaon. The regions which have an altitude of 5000 ft. (1500 meters) or more have snowfalls in winter. Rains generally start in the middle of June and continue up to middle of September with heaviest rainfall in July and August, there are some winter rains also.

It is only during the last eight years that virus diseases of fruit trees have been recognised in this area but the actual work was started in 1955. Till now the following diseases have been recognised:

Plum line pattern, Apple mosaic, Peach mosaic, Rubbery wood of apples, Ringspot of apricots, Rubus mosaic and mosaic of figs. This year one of us (N.S.B.) has recognised pear mosaic and almond mosaic also.

Plum line pattern disease

The disease has been found occurring naturally on different plum varieties viz. Green gage, Japanese plum, Maynard, Ramgarh Maynard and Sharpes' early. Ramgarh Maynard and Japanese

plum showed incidence of disease up to 80 per cent while it varied from 35 per cent to 50 per cent in other varieties.

The symptoms of this disease are confined to foliage and consist of yellow or creamy white patterns on the leaves emerging in the spring. Usually the veins are pale yellow scattered like a network. Later on the entire leaf becomes chlorotic. This pattern may occur over the whole leaf surface or may be confined to one half or merely a portion of a leaf. In addition there is bright yellow wavy pattern resembling an oakleaf pattern.

Chlorotic spotting appears as light green, yellow or creamy white spots on leaves. Those spots may be few or numerous and may be scattered evenly over the surface or grouped together in form of large chlorotic areas.

The intensity of symptoms varies from season to season. Marked symptoms appear on young leaves of plums formed in spring. These are more pronounced during summer but become less distinct in late summer and early autumn till they are masked all together. The symptoms vary not only in intensity but also in pattern changing from yellow vein clearing in spring to oak leaf type in autumn. There is a marked change in the colour of the leaf changing from deep yellow to creamy white and later to rusty brown.

Usually the symptoms are found on leaves formed during spring and early summer but are absent on others formed during autumn.

HOST RANGE AND VARIETAL SUSCEPTIBILITY

Plums. Four varieties of plums viz. Chaubattia Maynard, Ramgahr Maynard, Sharpes' early and Green gage got infected on inoculation but varieties Czar, new Plum and Plum I. showed no infection.

Peach. The disease was transmitted to peach seedlings and varieties Alexander, Early River, Hales' early and Red Nectarine but not to Duke of York. There are two types of symptoms exhibited by the seedlings. Some of the infected leaves showed symptoms early in spring as irregular wawy bands which tend to be ill defined with the advance of dry summer. These wawy bands sometimes form an oak leaf pattern.

The other symptom on the leaves is yellow vein chlorosis which may occur either on a portion of the leaf or over the entire leaf surface. These symptoms remain conspicuous till May, when they begin to fade and the entire surface of the leaf becomes pale yellow. The edges of the infected leaves become red.

Oak leaf pattern so common in plums is not prominent in infected leaves of most peach varieties. As the disease advances the leaves show rusty brown colour and distorted edges.

Apricot. Eight varieties viz. Frogmore early, Kaisha, New large early, Moorpark, Royal, Shipley's early and St. Amroise were inoculated with buds taken from diseased scion of Ramgarh Maynard. Budding was successfull in all varieties but the symptoms of the disease appeared only in Moorpark. Symptoms appeared in spring as small irregular chlorotic flecks and faint vein banding. Some of the leaves show irregular pale green spots. Symptoms remain prominent only in spring but tend to disappear in summer.

So far no transmission has been obtained in varieties of apples, four cherry varieties viz. Bedford prolific, Black heart, Late Duke, White heart; wild Cherry *Prunus Puddum* and wild pear *Pyrus Pashia.*

Attempts were made to inoculate several herbaceous plants by rubbing the sap obtained from infected leaves of Ramgarh Maynard, using carborundum powder but none of the following plants got infected:

Nicotiana tabacum, Nicotiana glutinosa, Cucumis sativus, Vigna sinensis, Vicia faba, Datura stramonium, Nicandra physaloides, Lycopersicum esculentum, Capsicum annuum, Gomphrena globosa, Phaseolus vulgaris, Chenopodium amaranticolor.

MOVEMENT OF THE DISEASE IN THE PLANT

Line pattern disease of plums is readily transmissible to peach seedlings by bud inoculation or grafting. In one experiment the behaviour of the disease was very inconsistent. Out of 30 seedlings inoculated from buds taken from diseased Ramgarh Maynard variety, only 23 seedlings showed symptoms and no symptoms

appeared in the remaining 7 seedlings. In 4 seedlings symptoms appeared even though the buds had collapsed.

In another experiment seven seedlings where no symptoms appeared in the previous year were reinoculated with fresh buds from infected plants. This time symptoms appeared in all showing thereby that probably the virus was not present in the buds in the first year.

To ascertain whether the buds from different branches of infected trees carry virus infection, an experiment was designed with the following treatment:

A. Buds taken from axils of leaves showing symptoms.
B. Buds taken from same budwood stick as (A) but from axils of leaves without any symptoms.
C. Buds taken from axils of leaves on a branch with all apparently healthy leaves.

Budding was done on healthy peach seedlings in the month of September and transmission of the disease recorded during the early spring. The results are given in table I:

Table I. Line pattern disease transmission by buds taken from axils of symptomless leaves and of those showing symptoms

	Buds from	No. of trees budded	No. of trees with symptoms	No. of trees without symptoms
A.	Axils of leaves showing symptoms	30	19	11
B.	Axils of leaves without any symptoms.....	30	14	16
C.	Axils of leaves without symptoms (apparently healthy).....	30	3	27

To confirm whether the plants which produced no symptoms in the above experiment carry any virus, buds from these plants were inoculated to healthy peach seedlings. None of them showed any symptoms showing them to be virus free.

From the results obtained above it is clear that all the buds do not carry infection and that there are apparently healthy leaves which carry the virus symptomlessly.

The disease is common in varieties Delicious, Jefferson, Jonathan, King, Russet, Spitzenburg and Tompking, the incidence varying from 20 per cent to 40 per cent while other varieties viz. Gloria Mundi, Red Astrackan, King David and Green George showed infection less than 20 per cent. Infected trees exhibit the following types of symptoms:

a. Chlorotic flecks as numerous small faint yellow chlorotic flecks scattered irregularly.

b. Large chlorotic areas as yellow creamy white areas which sometimes coalesced giving rise to larger chlorotic areas extending over two or more of the interveinal areas.

c. Vein banding: yellow coloured bands develop adjacent to the veins. These bands form a network pattern. They may occur over the entire leaf surface or be confined to a portion of a leaf.

d. Necrotic areas: Rusty brown necrotic areas appearing as large chlorotic patches. Sometimes necrosis develops from tips of the leaves and extends towards the midrib and the side veins.

The above mentioned symptoms differ in their character from season to season. First symptoms appear on young leaves in summer as small, pale yellow chlorotic flecks, large chlorotic areas and vein banding. Later in the season, the yellow colour of the infected leaves fades to creamy white in autumn. Leaves with large chlorotic areas or vein banding frequently develop necrosis. Diseased leaves tend to fall off much earlier than the healthy ones.

SENSITIVITY OF DIFFERENT VARIETIES TO SEVERE STRAIN

When buds were taken from the branches of a severely infected apple tree with buds from axils of leaves showing severe symptoms, varieties showed different reactions as given in table II.

Table II. Reaction of different varieties by buds taken from axils of leaves showing symptoms

Variety	Plants inoculated	Plants with severe sympt.	Plants with mild sympt.	Plants without sympt.	Reaction
Bramley's seedling	6	6	0	0	Severe
Cox's orange Pippin	4	0	2	2	Mild
Delicious	5	1	3	1	Severe, mild
Golden Delicious	4	0	1	3	Mild
Jefferson	5	5	0	0	Severe
Jonathan	5	3	2	0	Severe, mild
Rymer	5	2	3	0	Severe, mild
Sturmer Pippin	6	0	2	4	Mild
Sykes House Russet	5	0	4	1	Mild

Cross protection tests

The above experiment gave indications of the presence of two strains. An experiment was, therefore, set up to confirm their presence and to see whether the mild mosaic strain would protect the plants against the severe mosaic strain.

In one experiment seven plants of variety Rymer were inoculated with the mild strain. When the symptoms had appeared, five of them were reinoculated with buds taken from the axils of leaves showing severe symptoms.

In the second experiment buds from plants showing mild and severe symptoms were inoculated to four healthy Rymer plants.

It was found that all five plants reinoculated with the severe strain continued to show only mild symptoms. Healthy plants inoculated with the severe strain produced severe symptoms and those inoculated with the mild strain gave only mild symptoms indicating thereby that the mild strain prevented the multiplication of the severe strain.

Distribution of apple mosaic virus in host tissue

Results so far obtained have shown that the virus transmission is not consistent even if the bud and graft unions are successful. Inconsistency is not related to graft taken as there are trees with-

out symptoms where grafts were still alive and some trees developed symptoms on which the graft finally failed.

When inoculations were done with the buds taken from severely infected apple trees bearing leaves with and without symptoms, it was found out that all the buds from an infected branch do not carry virus infection and that the virus is not fully systemic.

Host range

Attempts to transmit the disease to peach seedlings and peach varieties viz. Alexander, Carmen, Chaubattia red, Duke of York, Early River, Hales' Early and Red Nectarine failed.

Peach mosaic

2 peach trees of Golden peach varieties in Krishnavilla orchard at Shyamkhat showed abnormal symptoms. Infected trees were dwarfed and profusely branched.

Leaves from infected trees showed fine vein chlorosis with yellow mottling which was conspicuous during spring. A few of the infected leaves are pale yellow in colour and narrower in shape. No colour break in petals was seen, probably the variety being a small flowered one. Fruits from such infected plants were bumpy and cracked in appearance.

The disease was succesfully transmitted to peach seedlings and to varieties Carmen and Red Nectarine, but not to apple seedlings. Infected leaves of peach show a mosaic pattern and are narrower in shape.

HEAT TREATMENT

It is now well known that if plants infected with line pattern of plums or mosaic of apples are kept at 37°C for 3-4 weeks, healthy buds or grafts can be obtained from rapidly growing young shoots of such infected plants. An experiment was done to see the effect of hot water treatment.

A set of twelve diseased seedlings and twelve cuttings 0.3 cms to 0.5 cms in diameter and 8-10 cms in length were immersed in water held at three different temperatures viz. 27°C, 37°C and 47°C for ten minutes daily for four consecutive days in the months of November and December. After treatment they were put in a

basin and their roots or cut ends were immersed in cold water. After four days the seedlings were potted and cuttings grafted to healthy seedlings. The results obtained are given in table III:

Table III. Heat treatment of infected seedlings and cuttings

Temperature	Treatment on	Plant	No. of seedlings/ cuttings used	No. survived	No. of plants with symptoms	No. of plants without symptoms
27 °C	Seedlings	Peach	12	10	9	1
		Apple	12	12	12	0
	Cuttings	Peach	12	10	8	2
		Apple	12	8	6	2
37 °C	Seedlings	Peach	12	11	3	8
		Apple	12	7	2	5
	Cuttings	Peach	12	7	1	6
		Apple	12	5	2	3
47 °C	Seedlings	Peach	12	0	0	0
		Apple	12	0	0	0
	Cuttings	Peach	12	0	0	0
		Apple	12	0	0	0

The above results show that the virus gets inactivated in most cases when seedlings and cuttings are treated for 10 minutes at 37°C for four consecutive days.

This method, however, did not prove to be a useful method for propagation of the healthy plants because such treated buds, grafts or seedlings show poor growth in later stages of their development.

DISCUSSION

BERNHARD: Have you noted a special aspect of the trunk of the trees infected with peach mosaic?

BHARGAVA: No!

CROPLEY: In the heat inactivation experiments were the whole plants used, or only certain buds of the plants?

BHARGAVA: Whole plants were used.

GUALACCINI: Did you in your inactivation experiments get inactivation in some branches only or in every branch?

BHARGAVA: Only partial inactivation (cure) was obtained.

JORDOVIC: Have you found any symptoms on fruits from trees infected by ring pox of apricot?

BHARGAVA: Not so far.

Das Vorkommen von Viruskrankheiten im Österreichischen Obstbau

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Um das Bild über das Auftreten von Viruskrankheiten im mitteleuropäischen Obstbau zu vervollständigen, möchte ich Ihnen heute im Rahmen dieses Kurzreferates über jene Virus- beziehungsweise viroiden Erkrankungen berichten, die bisher in Österreich nachgewiesen oder beobachtet wurden. Da wir uns eben erst bemühen, eine Sichtung der vorhandenen Virosen vorzunehmen, sind die Ihnen nun mitgeteilten Befunde keineswegs vollständig.

An sich spielt der Obstbau in Österreich nur in bestimmten Gebieten eine wirtschaftlich bedeutendere Rolle. Die wichtigsten Obstbaugebiete liegen im Osten und Südosten des Landes. Kernobstbau wird vornehmlich in den hügeligen Teilen der Steiermark und in Voralpengebiet Niederösterreichs betrieben. Von den insgesamt 9,590.000 im Ertrag stehenden Apfel- und den 4,802.000 Birnenbäumen Österreichs (Zählung von 1958) entfallen auf dieses Gebiet 5,187.000 beziehungsweise 2,100.000 Stück.

Beim Steinobst sind vor allem Aprikosen (660.000 Stück) und Pfirsiche (436.800 Stück) von Bedeutung. In den letzten Jahren sind im Donautal oberhalb von Wien zahlreiche Aprikosen- und im Süden der Steiermark (in der Nähe der jugoslawischen Grenze) nach modernen Gesichtspunkten arbeitende Pfirsichanlagen entstanden. Desgleichen erlebte der Beerenobstbau durch die Errichtung vieler Neuanlagen von Schwarzen Johannisbeeren einen fühlbaren Aufschwung. Erwähnenswert wäre noch ein geschlossenes Erdbeeranbaugebiet im Burgenland (nahe der ungarischen Grenze), in dem im Jahresdurchschnitt 5.000 t Erdbeeren (fast ausschließlich der Sorte Mme. Moutôt, die für den

Anbau im pannonischen Klima dieser Gegend gut geeignet ist) produziert werden.

Die größten Neuanlagen und alle 199 Markenbaumschulbetriebe Österreichs werden seit einigen Jahren durch Pflanzenschutzkontrollorgane auf sichtbaren Virusbefall untersucht. Latent auftretende Virosen konnten bisher nicht berücksichtigt werden. Je nach Obstart und Sorte waren die verschiedenen Viruskrankheiten in den einzelnen Betrieben in sehr unterschiedlicher Stärke vorhanden. Nur in Ausnahmsfällen betrug der Befall – etwa durch Apfelmosaik oder line pattern – mehr als 5 Prozent. Meist lag er weit unter 1 Prozent.

Im folgenden möchte ich Ihnen eine kurze Zusammenstellung der in Österreich bisher festgestellten Virosen und der Sorten, an denen diese vorkamen, geben:

A. Kernobst

Apfel: Mosaik.

Beobachtete Symptome: Sowohl schwaches als auch schweres Mosaik und Adernmosaik. Symptome wurden am häufigsten bei der Sorte Golden Delicious gefunden. Goldparmäne und Ingrid Marie waren vereinzelt befallen, James Grieve, Jonathan und Bohnapfel wiesen nur selten Symptome auf.

Flachästigkeit (flat limb): Verhältnismäßig häufig.

Beobachtete Symptome: Abplattungen, Rillenbildungen, Eindellungen an Ästen und Stämmen. An alten Gravensteiner-Standbäumen Absterben von Rinde und Kambium und auch ganzer Äste. Symptome am häufigsten bei Gravensteiner, ferner bei Ontario (bei diesem treten vor allem Abflachungen auf) und Abondanza. Symptome sind kaum im Folgejahr der Pfropfung zu erkennen. Sie werden erst nach 3 bis 4 Jahren deutlich.

Viröse Triebsucht (Proliferation disease): In einzelnen Ertragsbetrieben vereinzelt vorkommend.

Beobachtete Symptome: Vorzeitiges Austreiben der Seitenknospen, besenartige Verzweigung. Winkel zwischen Haupt- und Nebentrieb sehr klein, starke Vergrößerung der Stipulae, kleine Früchte mit langem Stiel, keine Chlorose oder Rotverfärbung der Blätter. Die Triebsymptome sind 1 Jahr nach der Pfropfung fest-

Fig. 1. Flachästigkeit (flat limb)

stellbar. Befallen waren vor allem die Sorten Boskoop, Berlepsch und Golden Delicious, selten auch Goldparmäne.

Birne: Steinfrüchtigkeit (stony pit): Vereinzelt an Bosc's Flaschenbirne. Beobachtete Symptome: Dunkelgrüne Flecke an der Schale junger Früchte. Später an diesen Stellen Eindellungen und Deformationen. Im Fruchtfleisch Steinzellennester. Blattsymptome wurden nicht gefunden.

B. Steinobst

Kirsche: Ring- und Bandmosaik (ring mottle): An Vogelkirschen (*Prunus avium*) verbreitet, auch an Süßkirschen vorkommend.

Beobachtete Symptome: Blaßgrüne Ringe und Bänder in verschiedener Anordnung, gelegentlich auch mosaikartige Sprenkelungen an den älteren Blättern.

Zwetschke: Bandmosaik (line pattern): Häufig.

Beobachtete Symptome: Gelbgrüne Ringe, zickzackförmige Bänderungen, Eichenblattmuster, bei der Sorte Magna glauca auch Symptome in Form kleiner Mosaikfleckchen vorkommend. Befall wurde bisher an den Sorten Bühler-, Ersinger-Frühzwetschke, Wangenheimer, Hauszwetschke und Magna glauca gefunden.

Pockenkrankheit (plum pox): Bisher vereinzelt in Siedlergärten an Bosnischer Zwetschke beobachtet.

Beobachtete Symptome: Zuerst Ringmuster an den Früchten, später Verkrüppelungen derselben und im Fruchtfleisch Gummibildung, vorzeitiger Fruchtfall, chlorotische Blattmusterungen.

Triebstauchung (prune dwarf): Einmal in Vorarlberg an Italienischer Zwetschke nachgewiesen.

Beobachtete Symptome: Blätter schmal, klein und etwas gewellt, Internodien verkürzt.

Pfirsich und Aprikose: Bandmosaik (line pattern): Verhältnismäßig häufig.

Beobachtete Symptome: Ähnlich jenen bei Zwetschke.

C. Beerenobst

Himbeere: Adernchlorose (vein chlorosis): An diesjährigen Trieben der Sorte Romy häufig.

Beobachtete Symptome: Aufhellung der Nervatur und unmittelbar angrenzender Gewebestreifen.

Erdbeere: Kräuselkrankheit (severe crinkle): Vereinzelt an der Sorte Mme. Moutôt auftretend.

Beobachtete Symptome: Blattkräuselungen, Verkleinerung einzelner Teilblättchen, gelegentlich punktförmige Aufhellung in der Blattlamina. In den Befallgebieten tritt der Vektor *Passerinia fragaefolii* ab Mitte Mai auf.

Derzeit noch ungeklärte Fälle

1) Rasp leaf – Symptome an Kirsche. Bei diesen Blattspreiten verschmälert, schärfere Blattzähnung und Enationen entlang der Mittelrippen.

2) Äpfel mit Symptomen der Rauhschaligkeit (rough skin), Sorte Boskoop.

Fig. 2. Weissfleckigkeit der Kirsche (genetisch bedingt?)

3) Birnen (Sorte Präsident Drouard) mit vein yellow Symptomen an den tertiären Adern.

4) Birnen mit red mottle Symptomen.

5) Quitten mit Mosaiksymptomen und streifigen Blattaufhellungen.

6) Schwarze Johannisbeeren mit den Symptomen der Nesselblättrigkeit (reversion).

Häufige viroide Erscheinungen sind bei uns:

a) Panaschierungen bei Kirsche (Weißfleckigkeit), oft verbunden mit Blattkräuselungen im Bereich der Mittelrippe oder kahnförmigen Herunterbiegen der Blatträndер entlang derselben.

b) Sternfleckigkeit (asteroid spot) an Aprikosen, Myrobalanen und Pfirsichen, die als Saugschaden, hervorgerufen durch die ektoparasitische Gallmilbe *Vasates fockeui*, anzusehen ist.

Um eine Zunahme der Viruskrankheiten an Obstgehölzen möglichst zu unterbinden, sollen die Kontrollen der Baumschulen und Neuanlagen weiter intensiviert und fortgeführt werden. Es besteht ferner die Absicht – zumindest in den Großbaumschulen – nicht bloß wie bisher, visuell als viruskrank erkannte Bäume auszumerzen, sondern die Testung des Vermehrungsmaterials hinsichtlich latenten Virusbefalles generell einzuführen.

SUMMARY

Up to now the following virus diseases were demonstrated by grafting to occur in the Austrian fruit growing areas:

ON APPLE:

Mosaic. Observed symptoms: Mild mosaic, severe mosaic and vein-banding. Sporadic on the varieties Golden Delicius, Goldparmäne, Ingrid Marie, seldom on James Grieve, Jonathan and Bohnapfel.

Flat limb. Observed symptoms: Rills and flats on stems and twigs. Often on the variety Gravensteiner, seldom on Ontario and Abbondanza.

Proliferation disease. Observed symptoms: Early shooting of the buds, whitch's broom, abnormal enlargement of the stipules, excessive proliferation of the water shoots. Often on the varieties Belle de Boskoop, Berlepsch and Golden Delicious, seldom on Goldparmäne.

ON PEAR:

Stony pit. Observed symptoms: Dark green areas just under the epidermis of young fruits. Production of sclerenchyma cells inside the fruit. No leaf symptoms. Sporadic on the variety Bosc's Flaschenbirne.

ON CHERRY:

Ring mottle. Observed symptoms: Pale green to chlorotic rings or irregular pale areas on leaves of *Prunus avium.*

ON PLUM:

Line pattern. Observed symptoms: Irregular yellowish lines and "oak leaf" pattern. The following varieties were attacked: Bühler-, Ersinger- and Wangenheims Frühzwetschke, Hauszwetschke, Magna glauca.

Plum pox. Observed symptoms: Sunken dots, lines and rings on the fruit, chlorotic pattern on the leaves.

Sporadic on the variety Bosnische Zwetschke.

Prune dwarf. Observed symptoms: Leaves small and narrow, shortening of the internodes.

Once on the variety Italienische Zwetschke.

ON PEACH:

Line pattern. Observed symptoms: Like those on plum.
In nurseries. Variety unknown.

ON APRICOT:

Line pattern. Observed symptoms: Like those on plum.
In nurseries. Variety unknown.

In order to stop the spread of virus diseases, all plantations and fruit tree nurseries have during the last few years been checked for virus diseases by plant protection inspectors. For the future it is intended in Austria to test generally the stock material for latent virus diseases at least in the bigger fruit tree nurseries.

DISCUSSION

KEGLER: We also found development of spindels in flat limb diseased Gravensteiner, Signe Tillisch and Ontario. Histological investigations showed that in flat limb diseased and spindle-like parts of branches the same histological variation occur. In both cases only parenchymous cells were developed. Therefore the possibility exist that flat limb and spindle are caused by the same agent.

RØNDE KRISTENSEN: In Denmark we have found spindle symptoms in the variety Signe Tillisch and we have experimentally prooved this disorder to be perpetual in the graftwood or budsticks, but actual transmission has not been obtained so far.

BUCHWALD: What is the reason of Weissfleckenheit of cherries? Do you think it is a genetic disorder or could the symptoms be due to thrips damage?

VUKOVITS: The reason is not quite clear but apparently virus is not the cause as transmission experiments so far have yielded negative results.

The symptoms are certainly not caused by thrips but are most likely of genetic character.

SUTIC: What do you think about the origin of plum pox (sarka) in Austria? Has the disease existed for long time in the country? How old are the trees infected with plum pox?

VUKOVITS: Diseased material is probably imported from the neighbouring countries during the war. Up to now plum pox diseased trees have only been observed in the Vienna area and the infected trees are about 10-20 years old.

GRAM: The explanation of the isolated occurrence of plum pox in Vienna may be that fruit trees notoriously have been the object of smuggling and peddling across the borders of some European countries. The trees frequently are of an inferior quality.

KEGLER: Have you transmitted flat limb to Gravenstein and Ontario, and if so which variety shows the earliest and most distinct symptoms?

VUKOVITS: In our transmission experiments Ontario reacted with distinct symptoms 2 years after the grafting while the first symptoms on Gravenstein first were noticeable after 3 years.

SZIRMAI: What kind of testplants were used to diagnose the proliferation of apple tree?

VUKOVITS: The apple variety Belle de Boskoop was used.

Data Regarding Virus Diseases of Fruit-trees in the Roumanian People's Republic

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Until 2-3 years ago no great concern was shown in Roumania for the investigation of the virus diseases of fruit-trees. However, some mentions were made about the existence of virus diseases of fruit-trees in the publication "Starea Fitosanitara in Roumania" (Plant Disease Reporter in Roumania) as early as 1941 (8).

The most important virus disease of fruit-trees in the Roumanian People's Republic is the *plum-pox, Annulus Pruni* Christoff. The disease was described in Roumania as far back as 1922 by D. I. Stefanescu under the name of "degeneration of Agen plum" (8). This disease is spread all over the Balcan Peninsula, and was discovered in 1932 by Atanasoff in Bulgaria (2) and in 1936 by Yossifovitch in Yugoslavia (11).

In Roumania plum-pox occurs in most fruit-growing areas, but is most frequent in old orchards in the south and east of the country. Its presence has been reported in the following regions: Bucuresti, Ploiesti, Craiova, Galati, Bacau and Suceava. Until recently the disease has not been noticed in nurseries, but in 1959 a fairly powerful attack was identified in some nurseries in the Bucharest region.

The virus attacks plum, apricot and myrobalan. On all susceptible species the disease is characterized by the appearance on the leaf blade of light green areas in the form of stripes, rings or semicircles, which alternate with tissue of normal colour (fig. 1 and 2). The shape of the leaf remains unchanged except for apricot whose leaves become slightly wawed. On young plum fruit round, semicircular or ringlike blotches appear, dark green in colour and with aqueous appearance which later turn to redish, blue-violet or whitish, deeply penetrating the flesh of the fruit.

Inside the tissue of the flesh and on the stones, such brown spots appear too. The fruits of the varieties "Pruna de Bistrita" and "Vinata Romineasca" are falling prematurely and loose their

Fig. 1. Plum-pox symptoms on plum leaves

flavour so that they can be consumed neither as fruits nor as preserves.

On the apricot fruits, superficial blotches appear. They are of ringlike shape and lighter colour than the rest of the surface (fig. 4). On the stones ringlike and almost white spots appear also, strongly contrasting with the remaining surface of the pericarp (fig. 5).

The disease has but small influence on the general development

of the attacked trees, though, many years after the infection, their productivity gradually decreases, the growth retards, ending by a precocious drying up of some parts of the crown.

About the transmission of this virus disease of plum, Atanasoff (1) and Christoff (4) have shown that, beside transmission through vegetative propagation, an important role is played by

Fig. 2. Plum-pox symptoms on apricot leaves

the insect *Anuraphis padi (Brachicaudus helichrysi)*. Yossifovitch (11) has proved that in Yougoslavia the only transmission of the plum-pox is through vegetative propagation. As for the insect vector, the author is of the opinion that it is not responsible for the spreading of this virus.

Regarding the ways of spreading of this virus disease in our country, following is to be noted:

In 1952, in a region where plum-pox is very frequent, 26 plum

hybrids have been obtained. The seedlings of one year age raised in that area have been planted, in 1953, on an experimental field in Bucharest. Although those hybrids have never been submitted to any grafting or mentoring, half of them are attacked by plum-pox.

Fig. 3. Plum-pox symptoms on plum fruits

In the spring of 1958, ungrafted apricots (multiplied through seeds) were found infected with the virus disease in several localities in Moldavia. Generally, these apricot trees obtained through seed, showed very slight symptoms on their leaves, whereas the fruit and especially the stone bore pronounced symptoms (fig. 6).

These observations lead us to the supposition that beside transmission through vegetative propagation, this virus has also other

Fig. 4. Plum-pox symptoms on apricot fruits

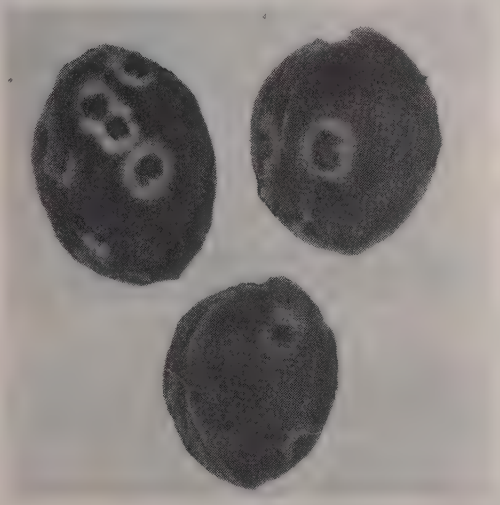

Fig. 5. Diseased apricot stones

possibilities of spreading: through the supposed vector which exists in our fauna, or more likely, through seeds. Observations made by Schuch (10) regarding the local apparition of that virus disease on a reduced number of specimens in Germany, point to the same conclusions.

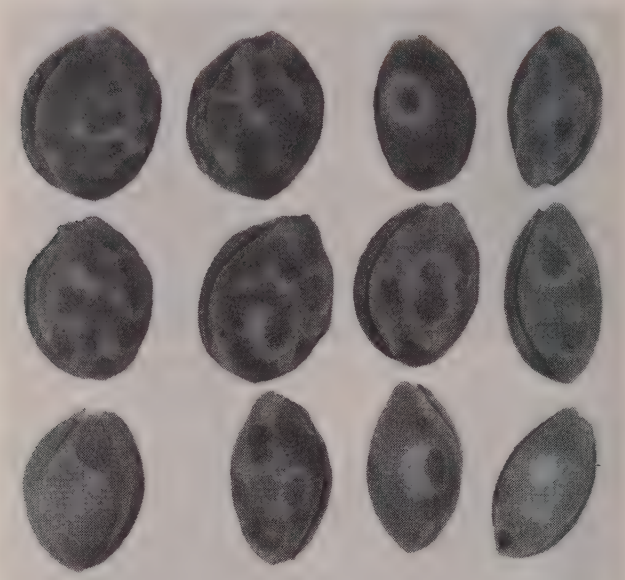

Fig. 6. Apricot stones from fruits grown on trees obtained from seed

This problem is now being experimentally investigated in Roumania.

The plum line pattern virus, Marmor lineopticum Cation, has been observed in recent years in some plantations in the Bucharest and Cluj regions; this disease is of much smaller economic importance than plum-pox. The characteristic symptoms are yellow-greenish rings which appear on the leaf blade, as well as light green stripes or lines in zigzags. Though the symptoms on the leaf are partly similar to those produced by plum-pox, the two virus diseases may easily be distinguished, as in case of line pattern virus there are no symptoms on the fruit which are most

characteristic for plum-pox. The two viruses also differ as regards the host range. While the plum-pox virus cannot be transmitted to peach, the line pattern virus can be transmitted to peach artificially as well as under natural conditions.

Fig. 7. Peach line pattern virus on peach leaf

The peach line pattern virus, Marmor lineopticum Cation was identified in Roumania only in the last years (5). Until now the disease has been observed only on some peach specimens in the Bucharest region. Symptoms appear on the leaves as green-yellowish or white-yellowish blotches which usually have the shape of

stripes going along the veinlets but sometimes may appear as rings or semicircles irregularly distributed on the surface of the limb.

Apple mosaic Marmor mali Holmes, was identified in Roumania in 1956 (9). Up to now the disease is little spread. The symptoms are confined to the foliage, consisting of light-green, yellowish or whitish spots. The spots may be either irregular in shape or circular. In some cases the ribs are discoloured while the tissue between them keeps its normal colour (fig. 8). Taking into con-

Fig. 8. Apple mosaic

sideration Schuch's classification (10) the strain of apple mosaic virus spread in Roumania, can be considered as less virulent, for no necrosis have yet been observed on the leaves.

The apple witch's broom virus (Proliferation virus). The symptoms of this virus have been observed only in a few apple trees from Transylvania. The characteristic of the disease consists in early growth of the buds on one year shoots and of the proliferation of stipules at the base of the leaves.

Pear stony-pit virus Marmor Pyri Holmes, is limited in our country. The first symptoms appear as irregularly distributed dark green areas under the epidermis of the fruit. The lack of growth in those areas and the rapid development of the surrounding tissues, results in deeply pitted or deformed fruit at maturity. Sclerenchyma cells are not uniformly spread in the flesh of the fruit, but concentrated in certain regions. On the leaves of af-

fected trees, slight discoloration is sometimes observed along the veinlets.

Apart from the virus diseases described above, we should mention that as a consequence of observations made and experiments carried out (6) the conclusion was reached that the symptoms known as asteroid yellow spot which are observed on stone fruits in nuseries and young plantations are caused in Roumania as well as in America (7) and Germany (3) by a species of gall-mite (*Vasates* sp.).

Excepting the plum-pox, we may conclude that, as compared to other countries, virus diseases of fruit-trees in our country occur in limited number.

It is also worth mentioning that in the R.P.R. virus diseases on the genus *Cerasus* are totally unknown. The peach, which in certain regions of the world is the most attacked species, presents in our country as yet a very reduced number of virus diseases.

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Report on Fruit-Tree Virus Diseases in Hungary

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Virus diseases of fruit-trees have been known for 12 years in Hungary.

The virus disease of apricot trees was found and described by us in 1948, when its occurrence was only sporadical in the country. At that time also some other virus diseases may already have

Fig. 1. Plum pox on older apricot trees. Leaf deformations, withering of the twigs; on the left: diseased trees; on the right: healthy trees

Fig. 2. Plum pox on apricot leaves; variety: Magyar Legjobb. Vein banding

appeared in our orchards, but their incidence was so sporadical as to escape the attention of the investigators. Very likely they were in a latent state without exhibiting any symptoms.

In the last years the apricot virus was spreading considerably, in consequence of which the problem became the object of thorough researches. These investigations revealed the identity of the virus disease occuring on the apricot tree with that described in Bulgaria by Atanasoff and Christoff under the name of *Prunus*

Fig. 3. Plum pox on the leaves of stocks; wild apricots. Ring pattern

virus 7 Chr. Annulus pruni Chr. or *Plum pox Atan.* The disease is also similar to peach-mosaic Marmor persicae Holmes, still it is somewhat different and may be a modified variant of the latter. While the symptoms appearing on the fruit are the same, transfer experiments to the peach tree show a different picture. The Elberta peach, which following the transfer was positive, did not show the typical protuberance and ring symptoms on the fruit even after 5 years, notwithstanding the appearence of a mosaic pattern on the foliage.

When the virus disease was recognized it only occurred in a single nursery, whereto it was introduced with propagative material from infected countries of south-east Europe 40 years ago. In this nursery an infection of 5 to 20 per cent could be ascertained on some varieties, such as Magyar legjobb, Hungarian best, Korai rózsa, Early rose, Borsi rózsa, Rose of Bors and Rakovszky.

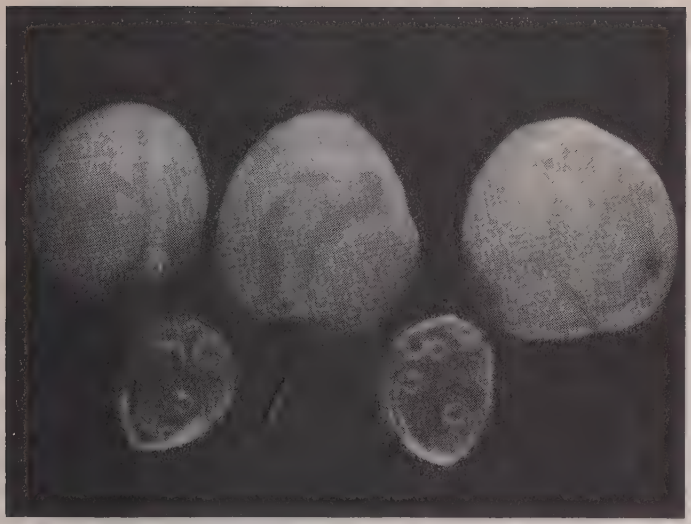

Fig. 4. Plum pox on apricot fruits, elevated blotches, and stones, yellow rings

As regards the other nurseries infection was only found in those, where the propagative material was procured from the former.

The virus also occurs on plum trees, but in our country it is more important on apricot-, than on plum trees, in which its distribution is not so wide as in the Balkan States. This is due to the different conditions of propagation, further to the entirely subordinate significance of the aphid vector, *Anuraphis padi*. In addition, the infection is slight on the plum stock and the insect vector is unknown for the apricot.

The virus is spreading only partly by seed but it is excellently transmitted with scions. When both possibilities of its transmission are taken into consideration, the conditions of a rapid distribution of the virus disease can be established.

After the heavy losses caused by apoplexy and damages due to the war, ten years ago, the production of fruit-tree seedlings proceeded at a forced rate. The seedling material indispensable for the growth of wild stock had to be obtained in a large part from the mixed seed material of mostly high bred commercial varieties available in the canning industry. Neither were scions

at our disposal in proper quantity, therefore they were necessarily collected from the seedling stock of the nurseries as well. A large part of the scions was most heavily infected precisely at that time. The seedling material obtained from canning industry, which was used temporarily for the growth of stock, contained sometimes 5 per cent infected seed. This explains why the occurrence of apricot virus in the nurseries rose to the maximum in the 1949-50 period. A strong positive correlation could be established between the virus infection of nurseries and the use for propagation of canning industry seedling material, respectively of infected scions from the nurseries. In such case the apricot virus infection of the nurseries jumped up to even 20 to 30 per cent on some varieties. Commencing from 1950 the quarantine regulations put in force improved the situation to such an extent that the virus infection of the most affected varieties declined to a small percentage.

In the nursery the diagnosis and characterization of the virus disease on two year-old scions does not meet any difficulties, but it proves to be more uncertain on wild stocks. In favourable cases virus symptoms of ring pattern can be recognized on infected stock already in the first year, but unfortunately the full infection becomes distinct only after 2 or 3 years, when the grafting of the stock has already taken place. In this case the diagnosis is made more difficult by the latency.

The presence of virus can be distinguished by the typical light-coloured rings on the stone fruit of apricot, thus the elimination of virus infected material can take place in due time. This is an essential measure, since from seedling material bearing virus-symptoms, 2 to 3 per cent infected wild stock will result.

Our wild apricot and myrobalan plants are not attacked as yet, thus healthy stocks can be grown from them.

The bulk of nursery infections originates from virus-infected scions deriving from uncontrolled mother trees. In such cases, in the year following the grafting, several plants are showing very poor growth with unsuccessfull sprouting due to infection. Though in favourable cases the eyes will sprout, stunted shoots will develop, the foliage of which exhibits the virus symptoms. In the seedling rows the occurrence of symptoms in groups is typical and

may be encountered in every case. This fact will assist to reveal the virus disease.

In the orchard the affected fruit-bearing trees are developing poorly and their duration of life is reduced. It is a characteristic feature that on trunks of three years old seedlings accessory shoots develop frequently, which will blossom abnormally early and set fruit as well, which however will not develop; sometimes the blossoms are asymmetrical.

In an experimental virus-infected planting it could be noted, in comparison with an adjacent healthy control, that after five years 16.6 per cent of the virus-affected trees were killed, whereas none died in the healthy control. The virus diseased trees had an average trunk circumference of 5.72 cm, while those in the control had 7.72 cm.

The virus diseased trees in a 19-year-old orchard lost their productivity in the age of 12 years, the number of branches drying out like those hit by apoplexy increased and abnormal shoots similar to witches broom developed. When the trees reached the age of 15 years, 90 per cent were killed.

The infection becomes heavier in densely planted orchards after a number of years, because the roots are getting in contact one with the other. It was observed that on trees infected via the root system, the signs of infection appear – both on the foliage and fruit – on the lower part of the crown. Hence it extends upwards towards the top of the tree. Parts of the tree not exhibiting any symptoms are already attacked. The virus spreads slowly in the tree, reaching the top only after one year. This fact was ascertained by sowing seedling material bearing the signs of ring pattern simultaneously with seed from the same material without any signs and the seedlings developed from both seed-lots were assayed for the virus. It was found that from seeds with ring pattern 2.2 per cent of the developed plants were bearing signs of the virus, but 1 per cent of the seedlings, which developed from the other lot, not bearing any virus-symptoms, was equally infected.

As regards the spread of the virus by other means than seed and eyes it should be noted that no mechanical infection can take place. Pruning experiments manually and with forceps

have demonstrated that the usual nursing works do not play any part in the virus transmission. Apparently a longer contact-connection, e.g. root-anastomosis, is necessary to bring about the transmission.

Experiments with vectors had similarly negative results. Apricot trees are not readily visited by aphids. In the autum season leafhoppers can be seen in large numbers, but they were not proved to act as vectors of the virus. Two neighbouring nurseries were held under observation, one of which was heavily infected with apricot virus, while the other was free of it. After several years of observation, no spontaneous virus-infection occurred in the latter, though only the propagative material was held rigorously in virus-free condition.

In addition to the plum pox virus of apricots a number of further symptoms was encountered in nurseries and orchards, which are typical for already known virus-diseases. Part of these is already identified while the diagnosis of another part is still in course.

Last year we discovered the Pfeffinger disease in one nursery, the origin of which could be traced back to an orchard farther off, from where scions were obtained.

The ring mottle of sweet cherries made a sporadical appearance already in several nurseries. It is undecided as yet, whether this pathological form represents a distinct virus or it is related with the Pfeffinger disease.

The Stecklenberger disease was found for the first time last year in a fruit-bearing sour cherry stand. The infected condition of older stock suggests that the infection is of a more remote date.

A number of sweet cherry trees was found to be suspect of leaf roll. The yellow leaf roll of peach is similar to the Swiss form, but seemingly it is connected at the same time with lime-chlorosis as well.

In addition to the viruses of stone-fruit apple mosaic was revealed in 1952, which fortunately is not distributed to a considerable extent as yet.

Fruit-trees suspect of apple witches' broom and chat-fruit are under investigation.

Finally mention should be made of some frequently occurring

Fig. 5. Apple mosaic, variety: Jonathan. Ochre yellow spots and sometimes deformations

viruslike syndromes, which are either known genetical disturbances, e.g. leaf mottle of cherry on the variety Germersdorf, or are not of virus origin, such as the asteroid spot of stone-fruit which was recognized as caused by mite injury.

It follows from the aforesaid that our most important duty consists in applying active control measures against the Prunus virus 7 of apricots by the employment of virus-free seedling material and eyes, further in revealing the new virus diseases and in their checking in due time. For this purpose suitable indicator plants were collected and propagated last year enabling us to carry out

Fig. 6. Panachure of cherry, variety: Germensdorfer. Mottling of genetic origin

test all over the country and in addition to this a "virological garden" was established which should serve for identification of fruit tree viruses on basis of comparisons.

The experiments mentioned were conducted and their results evaluated by the Hungarian Research Institute for Plant Protection and the Hungarian Research Institute for Horticulture.

DISCUSSION

Kegler: In connection to the information given by Dr. Szirmai I would like to add that Stecklenberger and Pfeffinger diseases are very widespread in Hungary.

Last year Dr. Nemeth from the Horticultural Research Institute in Budapest and I found orchards and nurseries, where 60-90 per cent infected plants occurred.

In transmission experiments, carried out in Budapest and Aschersleben, we established that these virus diseases are identical with those occurring in Eastern Germany and Switzerland.

Jordovic: What was the duration of your seed transmission experiments? And where did you keep your experimental plants?

Szirmai: The experimental plants were kept for seven years and outdoors.

Stout: Under what conditions did you obtain 1 and 3 per cent seed transmission?

Szirmai: 3 per cent transmission was obtained with seeds deriving from fruits showing ring spot pox symptoms and 1 per cent transmission was obtained with seeds from healthy appearing fruits from infected trees.

Sutic: In the case of positive seed transmission tests carried out outdoors in a 7 years period the results could possibly be due to some nature infection from uncontrolled sources.

Baumann: I should like to add, that Christoff in Bulgaria many years ago tried to transmit plum pox through seeds of apricots and plums but up to now with negative results.

Recent Progress on Fruit Tree Virus Diseases performed at Pavia, Italy

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We relate briefly the results on the experimental transmission of the fruit tree virus diseases that we are studying at the Botanical Institute of the University and the Cryptogamic Laboratory of Pavia.

Virus Diseases of Apple

1. *"Proliferation" ("Witches' broom")*
   The virus disease has been transmitted to different varieties and seedlings of apple. On quince seedlings we obtained dwarf growth, vein chlorosis and twisted leaves; leaf chlorosis on pear seedlings.
2. *Apple "Mosaic"*
   We obtained the transmission of the disease on different apple varieties and seedlings. Symptoms of "mosaic" appear on leaves of pear, quince, myrobalan, peach, plum and oriental persimmon.
3. *"Flat-limb"*
   We obtained the transmission of the disease on the apple varieties "Gravenstein" and "Abbondanza"; not on "Golden Delicious" and "Imperatore". Abnormalities appear on quince: Dwarf growth, vein chlorosis and twisted leaves. Withering and death of "Shirofugen", "Kwanzan" and "Montmorency" cherries. Dwarf growth and twisted leaves on peach seedlings, and abnormality on leaves of walnut.
4. *"Thumbmark"*
   "Ring-spot" on leaves of apple and pear. Dwarf growth, vein

chlorosis and twisted leaves on quince seedlings. Gum formation and dwarf growth on "Shirofugen" cherry. Not yet data on fruits.

5. *"Rubbery wood"*
   Transmission of the virus to apple "indicator" plants. Withering and death of "Montmorency" cherry.
6. *"Green crinkle" (?)*
   Dwarf growth, vein chlorosis and twisted leaves on quince seedlings. Not yet data on fruits.

Virus Diseases of Pear

7. *"Pear mosaic"*
   Transmission of the virus on pear and quince seedlings.

Virus Disease of Quince

8. *"Deformation, green spots and internal cork pitting on fruit"*
   Transmission of symptoms on quince fruits. On quince seedlings we obtained dwarf growth and intense leaf chlorosis. Leaf malformations on mazzard "F.12/1".

Virus Diseases of Sweet Cherry

9. *"Ring-spot"*
   Transmission on cherry virus "indicator" and on cherry seedlings.
10. *"Virus die-back"*. ("Moria-disease")
    Transmission of some symptoms on cherry virus "indicators". Dwarf growth, chlorosis and twisted leaves of peach seedlings.
11. *"Not yet identified" virus disease of sweet cherry*
    On cherry and on peach, we obtained a symptomatology very similar to the former virus disease.

Virus Disease of Sour Cherry

12. *"Tatter leaf"*
    We obtained "tatter leaf" and "leaf enations" on mazzard "F12/1" and on seedling cherries. "Mosaic" on "Lambert" and

"Napoleon" cherry. "Mosaic" and leaf enations on almond. Chlorosis and twisted leaves on peach seedlings. "Mosaic" on "Lombard" plum.

Virus Diseases of Flowering Prunus

13. *"Line pattern" of flowering cherry*
Transmission of the symptoms on cherry, "Shiro" plum, almond and myrobalan.
14. *"Not yet identified" virus disease of Prunus chinensis*
We obtained gum formation, withering and death of "Shirofugen" cherry. Dwarf growth and leaf necrosis of "Kwanzan" cherry. "Mosaic" and "ring-spot" on mazzard "F.12/1". "Line pattern" and "ring-spot" on myrobalan and on "Shiro"plum.

Virus Diseases of Plum and Prune

15. *"Line pattern"*
Transmission of the symptomatologi on plum, myrobalan, peach and almond. On mazzard "F.12/1" and on cherry seedlings we obtained also "necrotic spots".
16. *"Not yet identified" virus disease of Italian Prune*
In the experimental transmission we obtained "tatter leaf", "enations" and leaf malformations on mazzard "F.12/1" and on cherry seedlings. Probably associated with "leaf casting mottle", a virus-like disease of Prune.

Virus Diseases of Peach

17. *"Peach blotch"*
Transmission of symptoms on peach varieties "Elberta" and "Trionfo"; not on "Hale" and "Morettini 1". Leaf chlorosis on cherry seedlings. Dwarf growth, vein chlorosis and twisted leaves on myrobalan seedlings.
18. *"Rosette willow leaves"*
Transmission of symptoms on peach, apricot and on mazzard "F.12/1". On myrobalan seedlings dwarf growth, vein chlorosis and twisted leaves. Enations on cherry seedlings. "Willow leaves" and leaf malformations on apple seedlings.

Virus Disease of Almond

19. *"Almond mosaic"*

Transmission of symptoms ("mosaic" and "line pattern") on almond, peach, plum and myrobalan. "Line pattern" and "tatter leaf" on cherry seedlings and leaf malformations on mazzard "F.12/1".

Virus Disease of Hazel-Nut

20. *"Line pattern"*

We obtained the transmission of symptoms only on hazel-nut (seedlings and varieties).

DISCUSSION

Shay: What is the source of the quince seedlings used for indicating apple viruses?

Corte: I got the seedlings from professor F. Scaramuzzi, Florence.

Luckwill: You mentioned that rubbery wood caused wilting and death of Montmorency cherry. Was this on clonal Montmorency or on seedlings, and does Dr. Corte think that this reaction might be used to index apples for rubbery wood?

Corte: It was a clonal Montmorency from East Malling grafted on cherry seedlings. I suppose not that Montmorency would be a suitable indicator for rubbery wood as die-back occur not before 2-3 years after indexing.

Blumer: In the field the "Moria-disease" (virus die-back) in the Verona area is rather similar to Pfeffinger disease, although some symptoms are not so well expressed as in Pfeffinger. This may, however, be due to differences in the variety reaction.

Corte: In the experimental transmissions we have compared the "Moria-disease" of cherry with Pfeffinger disease, and the conclusion is that the latter is the most severe disease.

Development, Certification, and Maintenance of "Virus-Free" Nursery Stock

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The title of my paper contains the term "virus-free". This term is expressive and convenient, but it needs explanation. I would like to define it as meaning "apparently free from detectable viruses which are of concern in the plant material involved". It is a relative rather than an absolute term.

Practically speaking, there hardly can be nursery stock completely free of all viruses; and if it were possible to obtain and establish stock of such purity, its maintenance in the pure condition for any length of time probably would not be possible, or at least not commercially feasible, because of the hazards of natural spread. It follows, then, that at least some viruses, such as those of minor importance, or those of general distribution and not amenable to exclusion because of uncontrollable natural spread, necessarily must be tolerated in some degree.

Our principal effort is needed against the more serious and important viruses, and particularly against their introduction into a planting or into an area. These efforts also must sometimes be confined to developing and maintaining propagating stock free of only certain viruses.

Methods of developing virus-free nursery stock are of two kinds – (1) finding propagating material already free of viruses by selection and visual inspection of candidate source plants, followed by index or transmission test techniques to determine freedom from viruses, and (2) elimination of viruses from infected stock by certain techniques.

Visual inspection and indexing have been the most useful to us in finding clean propagating material (rootstock and top stock sources) of fruit trees and grapevines. We do need faster and more effective techniques. Such methods as those involving chemical tests, anatomical or cytological examination, chromatography, electrophoresis, the electron microscope, or serology, have specific limitations in the present state of our knowledge, although eventually we may find more very useful tools and techniques among these methods.

Considerable progress already has been made. Hutchins' test (2) has been very useful in the diagnosis of the phony disease of peach. The studies by Schneider (5,6) on changes in the phloem tissue at the bud union in the buckskin disease of sweet cherries growing on mahaleb rootstock, and also in the tristeza disease of orange trees growing on sour orange rootstock, have been very helpful in providing practical diagnostic laboratory procedures, particularly in the tristeza disease, where we use the method routinely as a diagnostic aid.

In grapevines, the studies of Gifford *et al* (1) on the presence of trabeculae or intracellular cordons, structures first reported by Petri in 1912 (4), which extend across the cavities of dead water-conducting cells of the xylem, have provided a means of laboratory diagnosis of the virus disease of grapevine known in California as fanleaf, in France as court-noué and dégénérescence infectieuse, in Italy as arricciamento or roncet, in Portugal as urticado, and in Germany as Reisigkrankheit.

Any program for finding clean propagating material by visual inspection and indexing on indicator plants must be planned on a broad basis of information so that a sufficiently wide host range of indicator plants can be determined upon and used to detect viruses which produce no symptoms in some hosts. As new information is developed, the program needs to be modified and kept up-to-date. It is highly important that there be sufficient research and information concerning the diseases, and the viruses which cause them, to provide a sound scientific working basis. The research must include development of suitable and practical methods for detecting, identifying, and evaluating the viruses, as well as methods of avoiding them. We have been extremely for-

tunate in that our movement for development of nursery stock free of serious virus diseases, and the research toward this end, have been organized on a regional and an interregional basis. There is frequent collaboration between the various research workers on fruit tree viruses in the different states, and between them and the workers of different countries, and rather extensive travel and field trips from one part of the country to another, as wel as between countries. Information accumulated after many years of extensive research by (these) cooperating workers resulted several years ago in our adoption of a list of eight indicator or "guinea pig" plants for use in indexing *Prunus* species. The indicator plants are:

Bing cherry on mahaleb rootstock *(Prunus avium L./P. mahaleb L.)*
Montmorency cherry *(P. cerasus L.)*
Shirofugen flowering cherry *(P. serrulata Lindl.)*
Nonpareil almond *(P. amygdalus Batsch.)*
Shiro plum *(P. salicina Lindl.)*
Italian prune *(P. domestica L.)*
Elberta peach *(P. persica (L.) Batsch)*
Tilton apricot *(P. armeniaca L.)*

Additional stone fruit indicator plants and some herbaceous indicator plants have been added to this list by some workers for research purposes, and certain modifications of the list are being used for testing different host species. The eight kinds listed were selected to provide an effective screen for detecting any serious viruses which might be present.

There have been various approaches to the problem of developing "virus-free" propagating stock by eliminating viruses from infected plants or plant parts. The techniques used have included physical or environmental factors, such as heat treatment; chemicals, as in chemotherapy; reproduction by seed in lieu of vegetative methods, so as literally to strain out the viruses during seed development; use of tip cuttings, in instances where the growing tip or shoot can outgrow the movement of the virus; propagation from numerous buds taken from an infected plant on the chance that one or more of the buds may have escaped invasion by the

virus and will grow into a clean shoot; and use of buds without woody tissue where the virus is present only in the xylem. Since the work of Kunkel (3) on eliminating the peach yellows virus from peach trees by heat treatment, numerous attempts have been made to use this method for other viruses. These attempts has been successful for some hosts and for some viruses. The chief limiting factor is in the nontolerance of the host to the treatment necessary to inactivate the virus. Chemotherapy is still highly experimental but may become useful. Many of the stone fruit viruses appear not to be seed transmitted, but, since stone fruit varieties do not come true from seed, the use of seed must be limited largely to rootstock propagation and breeding. In citrus, where nucellar seedlings can be used, the use of seed is very effective in the elimination of certain viruses. The nucellar seedlings, in citrus, do come true from seed. The use of tip cuttings to escape virus infection needs further investigation and development. The use of numerous buds from an infected tree, in an effort to find some buds which have not been invaded by the virus can be used successfully in instances where virus invasion of the host is not always complete. In phony disease of peach the virus is transmitted in grafts or buds only when they include xylem or wood, therefore, use of buds without woody tissue in the bud shield, as in June budding, is effective in escaping the phony disease virus.

Official certification programs now being administered by the California Department of Agriculture for producing, certifying, and maintaining "virus-free" propagating and planting stock of fruit crops are in operation for citrus, cherries, grapes, and strawberries. The program for citrus currently is primarily for one virus disease (psorosis or scaly bark) but there are plans to expand it to include other diseases. A program for peaches is being started. Other stone fruits and the pome fruits are on the waiting list pending further research and development of methodology. For the most part these programs are concerned with vegetatively produced stock, although rootstocks grown from seed also are involved. In the latter case, seed-transmitted viruses are an important consideration. All of these programs are required by California law to be on a self-supporting fee basis and all operate

under rigid regulations set up after public hearings as Sections in the California Administrative Code. They are conducted by the Bureau of Nursery Service in the Division of Plant Industry of the California Department of Agriculture, and the Bureau of Plant Pathology in the same Division provides technical guidance and develops methods. Work in the California Department of Agriculture on developing methodology for certifying that fruits, nut trees, and vine nursery stocks are free of serious virus diseases is carried on in a special federal-state financed project in collaboration with plant pathologists and horticulturists of the University of California, the United States Department of Agriculture, other states and countries, the counties in California, and nurserymen, orchardists, vineyardists, and private plant breeders. A program for the registration of cherry trees for use of rootstock and top stock sources was established in 1956 and was expanded in 1958 to include certification of cherry nursery stock grown from registered sources. Indexing of cherry rootstock and top stock sources for this program is done on five indicator hosts. These are Bing cherry on mahaleb rootstock, Montmorency cherry, Shirofugen cherry, Elberta peach, and Italian prune. It has been necessary to use this minimum list rather than the list of eight indicator plants previously mentioned, in order to bring the program into financial possibility for the nurserymen who must support the program with fees paid by them for the certification sevice. Three host indicators (Nonpareil almond, Shiro plum, and Tilton apricot) were omitted from the list of eight indicators in order to make the program financially possible. It was believed that the five indicators would serve the purpose at this stage of development of the program for commercial production of certified cherry nursery stock. It certainly is a tremendous improvement over the past when there was no organized program and when nurserymen obtained their propagating materials without sufficient knowledge of the presence of viruses in the materials.

The first step in the cherry program is the selection by visual examination of suitable candidate source trees. Budwood is taken from the selected tree and placed on seedling rootstocks for production of young trees, and, at the same time, tissue is taken

from the same selected trees for use in making index tests on the five indicator hosts. The young trees being produced are maintained under conditions to protect them from possible virus infection. The index tests on the five indicator hosts are observed for two successive growing seasons, and at least two visual inspections of the parent tree must be made in the same year that tissue is taken for indexing. Seed used to grow rootstocks for young trees which are to be registered must be from trees which have been tested in the same manner as top stock source trees so as to guard against seed transmission in the case of seed transmitted viruses.

To be eligible for registration a tree must be planted in a mother block, isolated at least one-half mile from any commerical planting of *Prunus spp.*, unless it is to be registered as a seed source only. The concern with seed source trees primarily is with seed-transmitted viruses. Each registered tree must be tested annually by indexing in Shirofugen cherry and must be given at least two visual inspections and found free from serious viruses or from symptoms or behavior characteristic of a serious virus disease. Periodic re-indexing on the five indicator hosts is required if the tree is to be retained in the program in a mother block for more than six years from the time of planting. Consideration is being given to extending this period to ten years.

To be eligible for certification, cherry nursery stock must be propagated from registered source trees or from approved propagating stock produced in an increase block. The increase block is a planting of yearling trees propagated from registered rootstock and top stock sources. Stocks to be certified must be given at least one visual inspection during the first growing season and at least two during the second season, and it must be inspected at the time of digging.

Any planting entered in the program must be kept in a thrifty growing condition, and pests must be kept under intensive control. Nursery stock will not be certified and seedling rootstocks will not be approved for propagating stock, if a planting is found infested or infected with any new pest or with a pest of limited distribution (unless not of economic importance) or with any of

the nematodes, *Pratylenchus penetrans, Pratylenchus vulnus,* or *Meloidogyne* spp., unless the infestation can be safely treated, or delimited and excluded from certification.

Any replanting, budding, re-budding, pruning, removal of nursery stock, or removal of trees in any planting must have the approval of the Department. Any plant found to be off-type or virus-diseased must be removed immediately from any planting, except that off-type branches of a registered tree may be removed instead of the entire tree. Trees or branches showing symptoms of a genetic disorder are considered off-type.

Cherry trees may be registered and assigned a registration number, or approved and tagged as approved cherry propagating stock, or as California certified cherry trees, when they have met the respective requirements of the regulations.

Six cherry varieties and two rootstock species are now eligible for certification. They are Bing, Lambert, Montmorency, Republican, Royal Ann (Napoleon), and Van for top stock varieties and mazzard and mahaleb for rootstock seed sources. It is anticipated that most of the cherry trees produced in California for 1961 planting will be certified. Fifteen nurserymen are participating in the cherry program, and there have been 132 top stock source trees and 13 rootstock seed source trees registered in 1960 (15 more rootstock seed source trees have been tested and are eligible). There were 94.000 cherry buds obtained in 1959 from registered sources for growing cherry nursery stock in 1960, and there were 7.662 cherry trees certified and sold as certified during the 1959-60 season. Sixty pounds of seed from registered mahaleb cherry seed source trees were produced in 1959.

The program for registration and certification of grapevines is very similar in principle to the cherry program. Grapevines or grapevine plantings may be approved and registered as rootstock and top stock sources for the propagation of nursery stock when inspected, tested and found free from serious virus diseases by procedures outlined in the regulations. Nursery stock, including buds, grafts, cuttings, rootings, and grapevines from these registered sources, may be certified and tagged as California certified grape nursery stock when all requirements of the regulations have been met.

Grapevine candidate rootstock and top stock sources currently are being tested for viruses by indexing in St. George, Emperor, and Mission varieties of grapevines. Since some grapevine viruses are retained in the soil, such as fanleaf and yellow mosaic viruses, and since at least one, fanleaf virus, is known to be transmitted by a nematode vector *(Xiphinema index)*, great care must be exercised in the selection of growing sites. Each planting must be located in an area where contamination by soil-borne grape viruses is not likely to occur from drainage, flooding, irrigation, or other means, and in an area where there is not likely to be damage from plant growth regulators. The planting must be at least 100 feet distant from any land on which grapevines have grown within the past ten years, or must be in a location approved by the Department. Treatment to eliminate soil-borne pests may be required. Recent experience has indicated that isolation from other grapevine plantings is advisable to reduce the hazard of natural spread by probable aboveground vectors. Registration sources of 25 grape varieties have been established and some nurserymen have produced plants for certification.

The problem of indexing citrus stock for detection of virus diseases is no less complex or difficult than in the case of stone fruits and pome fruits. If all of the known and suspected viruses are to be considered in extending the citrus program to include more than psorosis, a considerable number of indicator host plants (seedlings and top stock – rootstock combinations) must be used. More than two dozen such indicators are being used in the present program for indexing imported citrus propagating stock. These have ben selected to represent, for the most part, the commercial top stock and rootstock combinations being grown in California, so that any virus capable of injuring any of our generally used top stock and rootstock combinations, if present in the imported material in latent form, could be detected on one or more of the indicators. Had such a program been in operation at the time that the tristeza virus first was introduced into the United States, probably in a symptomless carrier, its presence could have been detected in the indexing of the imported stock. We know now that the tristeza virus, which is generally present in Meyer lemon plants, must have been present in the Meyer lemon

stock which entered California in 1908 from China. We are finding the virus to be present in certain sources of Satsuma orange trees, and occasionally in other imported citrus kinds and varieties.

In preparation for adopting an official program for peach stock, we have started indexing tests of 21 peach and nectarine varieties on the eight indicator hosts, previously mentioned. We must determine with experience whether all eight of the hosts will be necessary in our California program, and costs of the program may require that we reduce the number of indicator plants, if it is possible to do so without jeopardizing the program. In the beginning we are using all of the eight hosts.

The successful maintenance of fruit tree propagating material nursery stock in a "virus-free" condition primarily is a matter of protection of the stock from recontamination and reinfection. Such protection, which is not easy to accomplish, requires, in addition to over-all sanitary measures, careful maintenance of identity of the stock; a knowledge of the nature and biology of the host and its viruses on which a strong sanitary program can be based; and a knowledge of the necessary tools, facilities, techniques and over- all procedures necessary to carry out such a program. The required techniques and procedures must be closely and faithfully followed for they are based on fundamental physical, chemical, and biological laws. It is well established that these laws cannot be taken lightly or violated without penalty of failure.

The host plant to be maintained and protected must be well understood with respect to variety, general physical characteristics, physiology and growth habits, life history, methods of reproduction and propagation, environmental requirements, and the problems of commercial production and marketing. The viruses must be understood with respect to their methods of spread and infection, their environmental requirements and areas of occurrence and the factors required for their exclusion or for their suppression, eradication, or containment. In a consideration of how to protect clean stock from becoming infected, one must review the methods by which infection can take place. Among them are exposure to vectors which can carry inoculum and apply it effectively to the host; use of growing sites not suitable because of

location with reference to sources of inoculum, including symptomless carriers; growing in infested or infected locations or soil where a soil-retained virus may be present; and, finally, budding or grafting onto or with infected plant material.

Experience with our various registration and certification programs and our efforts to develop, establish, and maintain "virus-free" stocks has emphasized the necessity of strictly observing these basic principles of over-all sanitation and of taking definite measures for guarding against "accidents" and impredictable foreseen circumstances. The epidemiology or, if you please, the epiphytology of many viruses is not sufficiently known to permit the most intelligent and effective measures of protection from them. Sometimes we must adopt protective measures considered essential to be on the safe side in beginning a new program which later are determined to be more severe or extreme than really necessary. As new information is developed through research, these measures often can be modified, sometimes with the result of increased efficiency as well as less cost for a program, but until the new information is available, calculated risks must be kept to a minimum if failure is to be avoided. We must not, in our over-all operations, take unnecessary chances of contaminating clean stock by exposing it to an unclean environment or by grafting to infected understock or by trying to maintain the stock in areas where vectors are spreading inoculum, unless it is possible to protect the stock by controlling or excluding vectors. Human nature and economic factors, such as nursery practices and consumer and grower and marketing pressures and the urge to take unwise shortcuts for what at the time appears expedient, need to be guarded against. Above all, once a nucleus of clean stock, and particularly stock of a new variety, has been obtained through breeding or other expensive and time consuming methods, do not bud or graft all of the material onto an untested tree or rootstock which, although appearing visually to be healthy, may be infected with a virus which could cause the loss of the use of the new variety. Use a clean seedling instead for an understock. If a new variety is worthy of propagation, it is of prime importance that it be free of viruses at the outset of its development. As a protection against becoming infected through an understock, the original plant

should be retained separately in its clean state until its vegetatively propagated progeny is known to be established in a safe place on an understock known to be "virus-free". Protective maintenance of "virus-free" propagating sources largely is a matter of protection by isolation from natural spread. Growing grounds in which soil-borne viruses are present or which could become contaminated by drainage or otherwise from infected soil areas must be avoided.

One may ask, "What is the advantage of planting clean stock in an area where diseases are spreading naturally?". One answer is that there are many virus diseases for which there is little or no evidence of natural spread. But regardless of whether natural spread is occurring, it has been found, particularly in the case of certain viruses such as peach ring spot virus, that, when budwood is taken from "virus-free" sources and budded upon compatible and »virus-free« rootstocks, a higher budwood survival is obtained in the nursery row, more trees are produced per acre, and uniform stock of premium quality is the end product. Not only are production costs decreased from the nurseryman, but the quality and vigor of the trees he produces are increased, and the orchardist who buys and plants the nursery stock obtains a better quality of tree and eventually higher yields of fruit. The whole procedure also results in prevention of the planting of diseased trees and the introduction of viruses with them into new areas or plantings.

DISCUSSION

Dias: The endocellular cordons may be a good indication for virus infection in some varieties but not for all.

What should be done if a new virus is discovered in a nursery considered to be virus-free?

Stout: We can make use of endocellular cordons only when they are present, which means infection; but when they are absent we may not be sure of freedom from infection. We rely on indexing practically, rather than the absence of cordons.

If a new virus shows up in a nursery where the stock has been considered virus-free, the stock must be destroyed unless the new virus is of no economic importance, and the loss is to the nurseryman.

There is no refund of fees, because the fees were for the service rendered up to that point.

Attafuah: I agree there are many difficulties, generally speaking, in screening against viruses e.g. latency and seed transmissibility. Do you not feel that enough is known of certain particular viruses to enable special schemes to be drawn up for producing pure virus-free stocks, so that the only risk would be that of contamination later on?

Stout: Yes, I agree, so far as those particular viruses are concerned; and we are making use of such schemes for certain viruses.

Attafuah: When the question of mild virus was raised, you were not happy about its practical application. Is this due to the fear of possible mutation?

Stout: Yes, a mild form might mutate into a severe form, and a given mild form would not protect against all severe forms, and we could not rely on its protection.

Wolfswinkel: You mentioned that the offspring of a mother tree had to be propagated in isolation, a specified distance from other fruit trees. What is the procedure with the mother tree itself, growing amongst other, possibly infected trees?

Stout: Offspring of the mother trees do not require strict isolation in the nursery. The offspring trees are the ones sold under certification. Our requirements are that they be a separate planting sufficiently apart from other nursery stock so as to maintain their proper identity. The mother block contains only mother trees and such blocks must be located not less than 300 feet from any plant of *Prunus* and not less than one-half mile from any commercial planting of *Prunus*, unless enclosed by an insect-proof screenhouse.

Wolfswinkel: After selection, indexing and certification of a mother tree, is the nurseryman allowed to take budwood from this tree when he likes, or is it done under supervision of the inspection service to ensure that budwood is taken from the correct tree?

Stout: A nurseryman must give notice of the time he plans to collect budwood from his mother trees so that an inspector can be present to supervise the procedure. This ensures proper identification of all budwood.

Wolfswinkel: Where demand exceeds supply the nurseryman can sell as much uncertified stock as he can produce without difficulty and is therefore not interested to go to the expense and trouble of a certified scheme. Clearly an educational phase is required; can you suggest the lines on which this can be tackled?

Stout: It is important to understand that only one agency (the California Department of Agriculture) is authorized to certify the cherry stock. The buyer must learn that only stock which meets the requirements can be certified. Educating the buyer can be accomplished by meetings, by publication in trade journals, and through advertising by the nurserymen themselves. Both the nurseryman and the buyer of the certified trees learn by observation of the certified trees in the nur-

sery row and after planting in the orchard that they grow better and more uniformly than other stock.

WOLFSWINKEL: When it is known that a virus-free clone will be severely affected when attacked by a certain virus in a particular area, what is your opinion on the protection of this clone by infecting it with a mild strain of the virus before distribution to that area?

STOUT: Much experimental work has been done on the problem of a mild strain of virus protecting against a severe strain of the same virus. I know of no instance where practical application has been made of this phenomenon with plants. At the present time we know so little about plant virus diseases that one would have to use considerable caution before engaging in such a program. A given mild strain does not necessarily protect against all severe strains. Mild strains might combine and produce severe symptoms. Any mild strain might mutate into a severe strain.

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International Cooperation in Fruit Tree Virus Research

Report on discussion held 30th July 1960

By H. Rønde Kristensen

During the meetings of the last day of the Symposium (acting chairman: C.A.R. Meijneke) the following items were discussed:

1. Bibliography
2. Handbook of fruit tree viruses
3. Indicator list
4. Exchange of tested fruit tree material
5. Abstract exchange
6. Nomenclature
7. Next symposium
8. Proceedings

1. Bibliography

It was decided that one virus worker from each country should collect the bibliography from his own country and forward it to the committee for general distribution. Preferably a list of new publications should be issued every year. — The following "collectors" were suggested:

Australia and New Zeeland:	J. D. Atkinson[1]
Austria:	G. Vukovits
Canada:	M. F. Welsh[2]
Denmark:	H. Rønde Kristensen
Finland:	E. Tapio
France:	G. Morvan
Germany:	K. Schuch
Hungary and other Eastern countries:	J. Szirmai

1. Dept. of Scientific and Industrial Research, Private Bag, Auckland, New Zealand.
2. Plant Pathology Laboratory, Box 8, Summerland, B.C.

India:	K. S. Bhargava
Italy:	A. Canova
Japan:	Z. Hidaka
Jugoslavia:	M. Jordovic
Netherlands:	H. J. Pfaeltzer
Norway:	A. Bjørnstad
Portugal:	H. Dias
South Africa:	D. J. Engelbrecht
Sweden:	D. Lihnell[3]
Switzerland:	R. Bovey
United Kingdom:	A. F. Posnette
U.S.A.:	H. H. Thornberry[4]

2. Handbook

On behalf of the editorial committee Posnette suggested the following diseases to be included in the first handbook as well as the authors for each item.

Apple Diseases:

Chat fruit	by	Luckwill
Green crinkle		Kristensen
Rough skin		van Katwijk & Meijneke
Star crack		Cropley
Stiletten Krankheit		Hamdorff
Green dimple/ring blotch		Mezetti
Mosaic		Posnette and Cropley
Proliferation		Bovey
Rosette		van Katwijk
Flat Limb		Kristensen
Rubbery wood		Cropley
Swollen shoot		Bovey
Stem pitting and Latent viruses		Posnette and Luckwill
Virus-like disorders		Several contributors

Pear Diseases:

Stony pit	Kristensen
Mosaic (with ring pattern)	van Katwijk and Scaramuzzi
Red mottle	Cropley
Vein yellows	Posnette

3. Statens Växtskyddsanstalt, Solna 7, Sweden.
4. College of Agriculture, Dept. of Plant Pathology, University of Illionis, Urbana, Illinois, U.S.A.

Rough bark	Kristensen
Bark blister	Cropley
"Bristol Cross disease"	Canova
Latent viruses	Posnette and Cropley
Virus-like disorders, Rosette	Luckwill
Economic Importance	Schuch
Control Measures	Meijneke and Posnette

After some discussion it was agreed that anyone with particular experiences in any of the mentioned items should send contributions to the authors.

On suggest from G. Hamdorff it was decided not to have a special chapter on Stiletten Krankheit (virus nature not proved) but to put a few remarks about this phenomenon under the description of Rough Skin.

The publication of the handbook was discussed, and the committee was asked to investigate the relative costs of various methods when all the chapters were submitted by the authors.

3. Indicator list

A revised list of indicators of fruit tree viruses was suggested by the committee and with a few additions and alterations this list was accepted by the symposium members.

Copies of this list have already been distributed to all participants and to other colleagues working with fruit tree viruses.

4. Exchange of tested fruit tree material

It was agreed that the committee should work out a scheme for exchange of tested fruit tree material and the principles for this exchange was discussed.

5. Abstract exchange

It was recommended that each virus worker should distribute reprints of their own publications to all symposium members and furthermore to the Commonwealth Bureau of Horticultural and Plantation Crop, East Malling, Kent, England.

6. Nomenclature

Preservations of the names Eckelrader and Pfeffinger for the two rasp-leaf diseases of sweet cherry occurring in the Netherlands and Switzerland respectively, was recommended, because the proposed synonym (rosette) has been used for a distinct disease of sour cherry in the U.S.A.

7. Next Symposium

The participants accepted an invitation to have the next symposium in Italy — if possible in the beginning of of June 1962.

8. Proceedings

The proceedings of the symposium will appear in "Tidsskrift for Planteavl" and reprints will be available.

A Revised Standard Minimum Range of Indicator Varieties for Fruit Tree Viruses in Europe 1961

composed by the European Committee for Cooperation in fruit tree virus research.

Note: This list of suggested indicators will need to bee amended periodically as new information becomes available.
Alternative indicators are given in brackets.

Host and disease	Virus indicator	Where obtainable
Apple		
Rubbery wood	Lord Lambourne	1, 2, 3, 4,
Mosaic	Lord Lambourne	1, 2, 3, 4,
	Jonathan	2, 4,
Chat fruit	Lord Lambourne M 139	1, 2, 3, 4,
Proliferation	Belle de Boskoop	1,
Witch's broom	Gravenstein (= Graasten)	1, 2,
Rough Skin	Belle de Boskoop	1,
Star Crack	Cox's Orange Pippin	4,
Green crinkle	Guldborg	2,
Flat limb	Gravenstein (= Graasten)	1, 2,
Rosette	Belle de Boskoop	1,
Stem pitting	Virginia Crab	3, 4,
Other latent viruses	Spy 227	3, 4,

Host and disease	Virus indicator	Where obtainable
Pear		
Stony pit	Beurré Hardy	1, 2, 4,
	Bosc	6,
Mosaic	Beurré Hardy	1, 2, 4,
Vein yellowing	Beurré Hardy	1, 2, 4,
	(Bon Chrétien Williams)	4,
Blister bark	Bon Chrétien Williams	4,
Split bark	Bon Chrétien Williams	4,
Cherry		
Ring spot (including tatter leaf and ring mottle)	Mazzard F 12/1	4,
Rugose mosaic (rosette)	Mazzard F 12/1	4,
Rusty mottle	Mazzard F 12/1	4,
Leaf roll	Mazzard F 12/1	4,
Yellow mosaic (caused by plum line pattern virus)	Mazzard F 12/1	4,
Stecklenberger disease	Mazzard F 12/1	4,
Rasp leaf	Bing	4,
Pfeffinger	(Napoleon)	4,
Eckelrader	Bing	4,
	(Napoleon)	4,
Sour cherry yellows	Montmorency	1, 4
Little cherry	Van (leaf sympt.)	4,
	Sam (leaf sympt.)	7,
	Lambert (fruit sympt.)	7,
Plum		
Prune dwarf	Italien Prune	4,
	(Krikon)	7,
Line pattern	Peach seedlings	Locally
	(Mazzard F 12/1)	4,
	(Shiro plum)	4,
Ring spot	Mazzard F 12/1	4,
	(Peach seedlings)	Locally
Bark split	Cambridge Gage	1, 4,
Plum pox	Pozegaca	1, 5,
Peach		
Ring spot Line pattern	See plum and cherry	
Other diseases	Peach seedlings	Locally

Host and disease	Virus indicator	Where obtainable
Apricot Die back	Peach seedlings	Locally
	Apricot seedlings	Locally
Latent stone fruit viruses		
Ring spot strains	Peach seedlings	Locally
	Shirofugen	Locally
Green Ring Mottle	Kwanzan	Locally
Dusty yellows	Mazzard F 12/1	4,
Dark Green Mottle	Peach seedlings	Locally

LIST OF INSTITUTES WHERE INDICATOR MATERIAL CAN BE OBTAINED:

1. Plantenziektenkundige Dienst, Wageningen, Netherlands (C. A. R. Meijneke)
2. Statens plantepatologiske Forsøg, Lyngby, Denmark (H. Rønde Kristensen)
3. Stations fédérales d'assais agricoles, Changins s/Nyon, Switzerland, (R. Bovey)
4. East Malling Research Station, Maidstone, Kent, England (A. F. Posnette)
5. Institut za Voćarstvo, Cacak, Yugoslavia (M. Jordović)
6. Cornell University, Geneva, N.Y., U.S.A., (K. D. Brase)
7. Prosser Irrigation Exp. Sta., Prosser, Wash., U.S.A. (Paul Fridlund)

Research workers are requested to ask only for small quantities from which to propagate their own supply.

It should also be noted that the above mentioned indicators are not guaranteed free of all viruses and should not be used for general propagation in the nurseries.

Care should be taken not to bud or graft the various indicators directly onto the trees under test.

The Committee
h. t. A. F. Posnette
R. Bovey
C. A. R. Meijneke
H. Rønde Kristensen

INDHOLD

Proceedings of the Fourth Symposium on Virus Diseases of Fruit Trees in Europe. Lyngby 25.—30. July 1960

NIELSEN & LYDICHE (M. SIMMELKIÆR)
KØBENHAVN